Project ID 526

## Competitive Research Grant

# Sub-Project Completion Report

## on

# Studies on the species complex and their bio-rational based management of fruit flies infesting fruits and vegetables in Bangladesh

**Project Duration**

**April 2017 to September 2018**

**Entomology Division, Bangladesh Agricultural Research Institute**

**Submitted to**
**Project Implementation Unit-BARC, NATP 2**
**Bangladesh Agricultural Research Council**
**Farmgate, Dhaka-1215**

**September 2018**

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Citation
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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

Edited and Published by:

Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

***Acknowledgement***
The execution of CRG sub-project has successfully been completed by [insert org/inst/uni] using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thanks to the World Bank for arranging the grand fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

Published in: September 2018

Printed by: [Name of press with full address]

# Acronyms

| | | |
|---|---|---|
| PCR | : | Project Completion Report |
| BARI | : | Bangladesh Agricultural Research Institute |
| BARC | : | Bangladesh Agricultural Research Council |
| PIU | : | Project Implementation Unit |
| Ffly | : | Fruit Fly |

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# Executive Summary

Fruit flies, *Bactrocera* spp. (Diptera: Tephritidae) are among the most economically important insect pest in Bangladesh attacking a wide range of fruits and vegetables. Numerous fruit fly species constitute enormous threats to fruit and vegetable production causing both quantitative and qualitative losses. In Bangladesh, most of the reports on fruit fly pests reveal that mostly two species of *Bactrocera* are present in our country. *B. cucurbitae*, infesting 16 cucurbitaceous vegetables and another one *B. dorsalis,* infesting different fruits. But recent reports indicate that several other species are also present in our country and causing serious economic loss of both vegetables and fruits. Fruit fly species composition is not well documented in our country. On the other hand significant achievements have been done in developing and popularizing pheromone based IPM technologies against fruit fly in vegetables and fruits, but resurgence of new species are causing hindrance for its cost effective management. So, it is a need to identify the species complex, their host range and to develop their bio-rational based sustainable management. Keeping these views in mind, the present research work has been designed.

Collection of 10 species of fruit flies from 8 different locations using pheromone traps was done. Identification of the collected specimen using taxonomic keys through morphological variation 07 (seven) species of Fruit Fly was identified. *B. nigrofema* and *Ceratitis cosyra* has been recorded first time in Bangladesh. *C. capitata*, which is popularly known as Mediterranean fruit fly (Medfly) is one of the world's most destructive fruit pests, has been collected only from Rahmatpur and Barishal. However, molecular identification study through phylogenetic analysis of the isolates based on COI sequences revealed that there were four major species group viz. *Bactroera dorsalis* (13 isolates)*, B. tau* (8 isolates) and *B. cucurbitae* (4 isolates). Among them three group viz. *Bactroera dorsalis, B. tau* and *B. cucurbitae* were the most prevalent. The newly identified (morphological) the most destructive fruit fly, *Ceratitis capitata* was not confirmed by molecular identification. Based on COI (Cytochrome c Oxidase 1, a mitochondrial gene) sequencing, it was identified as *Bactroera tau. Bactroera dorsalis* outnumbered all other species and can be considered as the most prevalent species of fruit flies in Bangladesh followed by *B. cucurbitae*. *B. tau* also recorded throughout the year; however their number is less than those two major species. The highest numbers of fruit flies were recorded during hot and humid seasons, viz. during the months of June and July. On the other hand lowest fruit fly populations were recorded during cool and dry months, viz. December-January.

Development of bio-rational management packages against different species of fruit flies were done on guava, gourd and bitter gourd at Gazipur. On guava significantly lowest infestation 0.00% and highest healthy fruit yield 20.28 ton/ha was recorded from the bagging of fruits with polythene, followed by sanitation + attract & kill method. On gourd lowest infestation 3.17% and highest yield 22.70 t/ha was recorded from sanitation + attract & kill method + application of soil recharge treatment followed by Sanitation + attract & kill method, Sanitation + Pheromone (culure) mass trapping in water trap + Soil Recharge, However the highest MBCR was obtained from the Sanitation + Pheromone Mass Trapping treatments (5.33) followed by sanitation + attract & kill method (4.42). On bitter gourd significantly the lowest infestation 6.54% and highest yield 15.82 tons/ha was recorded from the Treatment comprising sanitation + attract & kill method followed by the treatment Sanitation+ Pheromone (culure) mass trapping in water trap, However the highest MBCR was obtained from the Sanitation + Pheromone Mass Trapping treatments (9.38) followed by sanitation + attract & kill method (8.44). Field validation of developed technologies were done one at Jessore and two at gazipur locations where the attract and kill based IPM technology recorded 72.3-84.2% less fruit infestation by fruit fly species complex resulting 39-5-52.0% yield increase of healthy fruits. Three field days were organized in the trial areas where 150 participants of different stakeholder were attended. One booklet has already been done on the “Attract and kill method” for effective fruit fly management and two scientific papers are in preparation to be submitted in the Bangladesh Journal of Entomology.

# CRG Sub-Project Completion Report (PCR)

## A. Sub-project Description

| | | | |
|---|---|---|---|
| 1. | **Sub-project title** | : | **Studies on the species complex and their bio-rational based management of fruit flies infesting fruits and vegetables in Bangladesh** |
| 2. | **Implementing organization** | : | Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur -1701 |
| 3. | **3. 1 Principal Investigator** | : | Dr. Syed Nurul Alam, Director, Planning and Evaluation Wing and Ex Chief Scientific Officer & Head, Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh, Telephone: 02-49270024, Mobile: 01711 907886, E-mail: alamsn09@gmail.com. |
| | **3. 2 Co-Principal Investigators** | : | 1. Dr. Md. Akhtaruzzaman Sarkar, Senior Scientific Officer, Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh, Mobile: 01556 300588.<br>2. Dr. Md. Muzahid-E-Rahman, Senior Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh, Mobile: 01717 434966. |
| 4. | **Sub-project budget (Tk)** | : | |
| | **4.1 Total** | | Taka 24,00,000.00 (Twenty four lacs only) |
| | **4.2 Revised (if any)** | : | Taka 22,43,362.00 (Twenty two lacs forty three thousands three hundred sixty two) only |
| 5. | **Duration of the Sub-project** | : | 17 months |
| | **5.1 Start date** | : | 17 April 2017 |
| | **5.2 End date** | : | 30 September 2018 |
| 6. | **Justification of undertaking the sub-project** | | Fruit flies, *Bactrocera* spp. (Diptera: Tephritidae) are among the most economically important insect pest in Bangladesh attacking a wide range of fruits and |

vegetables. Numerous fruit fly species constitute enormous threats to fruit and vegetable production causing both quantitative and qualitative losses. In Bangladesh, most of the reports on fruit fly pests reveal that mainly two species of *Bactrocera* are present in Bangladesh. *B. cucurbitae*, infesting 16 cucurbitaceous vegetables and the another one *B. dorsalis,* infesting different fruits. But recent reports indicate that several other species are also present in our country and causing serious economic loss of both vegetables and fruits. Fruit fly species composition is not well documented in our country. On the other hand significant achievements have been done in developing and popularizing pheromone based IPM technologies against fruit fly in vegetables and fruits, but resurgence of new species are causing hindrance for its cost effective management. So, it is a need to identify the species complex, their host range and to develop their bio-rational based sustainable management. Keeping these views in mind, the present research work were designed, which complied with the National Agricultural Policy, Sustainable Development Goal to ensure safe food and environment.

7. **Sub-project goal** **:** Sustainable management of fruit flies attacking different fruits and vegetable crops to increase yield and quality of those crops.

8. **Sub-project objectives** **:**
1. Identification and documentation of species composition of fruit flies infesting fruits and vegetables using morphometric and molecular tools.
2. Development of bio-rational management options of different fruit fly species.
3. Field validation of the developed technologies in the farmer's field.

9. **Implementing locations** **:** Gazipur, Jessore, Chapai Nawabgonj, Rangpur, Jamalpur and Khagrachari.

10. **Methodology in brief** **:**

The project targeted identification and documentation of species composition of fruit flies infesting vegetables and fruits using morphometric and molecular tools, development of bio-rational management technologies of species complex of fruit fly and validation of developed management options at the farm levels. Collection of fruit flies were started by using sex pheromone traps of 10 species of fruit fly, viz. *Bactrocera cucurbitae, B. tau, B. triyoni, B. zonata, B. papayae, B. dorsalis, B. nigrofema, Ceratitis capitata, C. rosa and C. cosyra* at 8 different locations. The morphometric identification as well as molecular identification study of the collected specimen and research on the development of management options was also been done at BARI Central Station, Gazipur.

There were 06 main activities of the project enumerated as follows (WP stands for work program):

WP1: Selection of locations, farmers, manpower etc.

WP2: Collection of fruit flies has been done by deploying sex pheromone traps of 10 species of fruit flies from 08 locations. The species and the locations are as follows:

| **Species (10)** | **Locations (08)** |
|---|---|
| *Bactrocera cucurbitae* | Gazipur |
| *B. tau* | Jamalpur |
| *B. triyoni* | Ishardi |
| *B. zonata* | Bogra |
| *B. papayae* | Rangpur |
| *B. dorsalis* | Jessore |
| *B. nigrofema* | Barishal |
| *Ceratitis capitata* | Khagrachari |
| *C. rosa* | |
| *C. cosyra* | |

Pheromone lures of 10 species of Russell IPM Ltd. UK origin were used for Fruit fly monitoring. Water traps were used for trapping the adult male fruit flies of different species. Number of trapped fruit flies were recorded at every three days interval. Trapped insects were preserved for further investigations especially for molecular identification. Monitoring of fruit flies started from August 2017.



Pheromone lures of ten species used for fruit fly monitoring

Pheromone traps on different crops at 08 locations

Morphometric and molecular identification of the collected species composition of fruit flies infesting vegetables and fruits were done.

WP3: Distributions of the identified species at all study locations were done.

WP4: Development of bio-rational based management packages against different species of fruit flies has been done on guava, gourd and bitter gourd at Gazipur.

WP 5: On-farm validation of the developed management packages has been done at Shreepur, Gazipur, Bagarpara, Jessore & Sadar, Chapainawabgonj.

WP 6: Awareness building of farmers and extension personnel by arranging three field days one at Sreepur and two at BARI campus on the developed technologies.

11. **Results and discussion**:

**Identification of the collected fruit flies:**

**Morphometric identification**

The morphometric identification study of the collected specimen has been done in the IPM Laboratory of Entomology Division, BARI, Gazipur. The specimens identified using taxonomic keys through morphological variation is as follows:

***Bactrocera dorsalis***: Most widely spread and a pestiferous tephritid fruit fly in the world as well as in Bangladesh. It is polyphagous pest and infest wide range of fruits during their mature stage. Morphologically it can be identified by its hyaline wing (no spots on the wing).

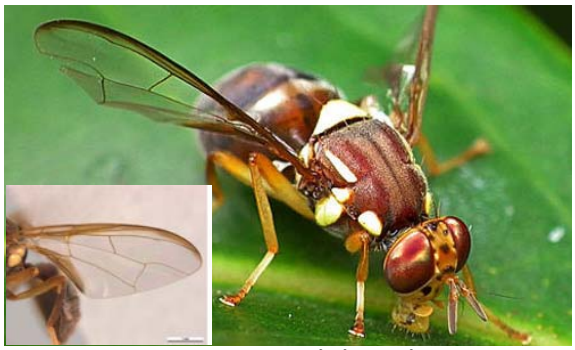
*Bactrocera dorsalis* adult male

*Bactrocera cucurbitae* adult male

***Bactrocera cucurbitae:*** A predominant and pestiferous tephritid fruit fly in the world as well as in Bangladesh. It is polyphagous pest and infest wide range of Cucurbitaceae vegetables during their early fruit setting stage. Morphologically it can be identified by its spotted wings.

***Bactrocera tau:*** Widely distributed in different Asian countries. Very destructive pest of Cucurbitaceae. Recorded in Gazipur, Jamalpur, Jeshore, Bogura and Khagrachari. Morphologically it can be identified by its typical wing venation.

*Bactrocera tau* adult male



*Bactrocera tryoni* adult male

***Bactrocera tryoni*** (Queensland fruit fly): Very destructive pest of citrus in Australia. It can infest more than 100 species of fruits and vegetables, especially apple, guava, mango, cucumber, tomato etc. It has been recorded in Gazipur, Jamalpur, Bogura, Rangpur and Khagrachari. Morphologically it can be identified by its typical wing venation.

***Bactrocera papaya:*** It attack a wide variety of fruit, have high reproductive potential, and are known invasive. It is mostly a pest of South East Asian region. In Bangladesh it was recorded in all locations except Rangpur.

***Bactrocera nigrofema:*** It attack a wide variety of fruit, especially olives. It is distributed throughout the world including South Asia. In Bangladesh it was recorded in Gazipur, Jamalpur, Bogura, Khagrachari, Jeshore.

*Bactrocera papaya* adults. Female with sharp ovipositor

*Bactrocera nigrofema* adult

***Ceratitis capitata,*** Mediterranean fruit fly (Medfly): The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the world's most destructive fruit pests. The species originated in sub-Saharan Africa. *C. capitata*, which is popularly known as Mediterranean Fruit Fly and considering as the devastating fruit fly species, has been collected from Rahmatpur, Barishal. The Mediterranean fruit fly attacks more than 260 different fruits, flowers, vegetables, and nuts. Thin-skinned, ripe

succulent fruits are preferred by the pest. Host preferences vary in different regions. Several species of cucurbits have been recorded as hosts of the medfly.

*Ceratitis capitata,* Mediterranean fruit fly (Medfly)

**Molecular identification of the collected specimens:**

**DNA extraction, PCR amplification and Phylogenetic analysis**

For the DNA extraction, total DNA was extracted from individual fruit fly adults by using a commercial DNA isolation kit (Promega, USA) with necessary modifications. The universal Cytochrome Oxidase I (COI) gene primers such as LCO1490 (5'GGTCAACAAATCATAAAGATATTG-3') and HCO-2198 (5'TAAACTTCAGGGTGACCAAAAAATCA-3') were used. The reaction condition was considered in the polymerase chain reaction as follows: 94°C for 3 min, followed by 39 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min and then a final incubation at 72°C for 10 min. Five microliters of each amplification mixture was verified by agarose (1% w/v) gel electrophoresis in 0.5X Tris-borate-EDTA (TBE) buffer. Molecular characterization of the samples were determined by the sequencing of COI gene by a company ($1^{st}$ Base Laboratories, Malaysia) followed by phylogenetic analysis. After sequencing of COI gene amplicons, homology was evaluated with NCBI BLAST tool for comparison and identification of the samples.

The PCR amplified products were purified using commercial kit, and then incubated at 37 °C for 60 min followed by 80 °C for 20 min. The nucleotide sequences were determined using dideoxy sequencing techniques at $1^{st}$ BASE Company, Malaysia (taken as commercial service). The COI sequences were combined using the Bioedit software, checked manually, corrected, and then analyzed using the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/) to identify the isolates. Phylogenetic analyses were conducted using the MEGA 7 program, and a neighbor-joining tree was constructed using the Kimura two-parameter model. The phylogenetic tree was generated using the most identical sequences of fruit fly available in the GenBank database. Confidence values were assessed from 1,000 bootstrap replicates of the original data.


Fig. 1. Phylogenetic tree based on Cytochrome Oxidase I (COI) gene sequences revealing the phylogenetic relationships among the *Bactrocera* species. The 27 isolates from this study are indicated in the tree with a black diamond.

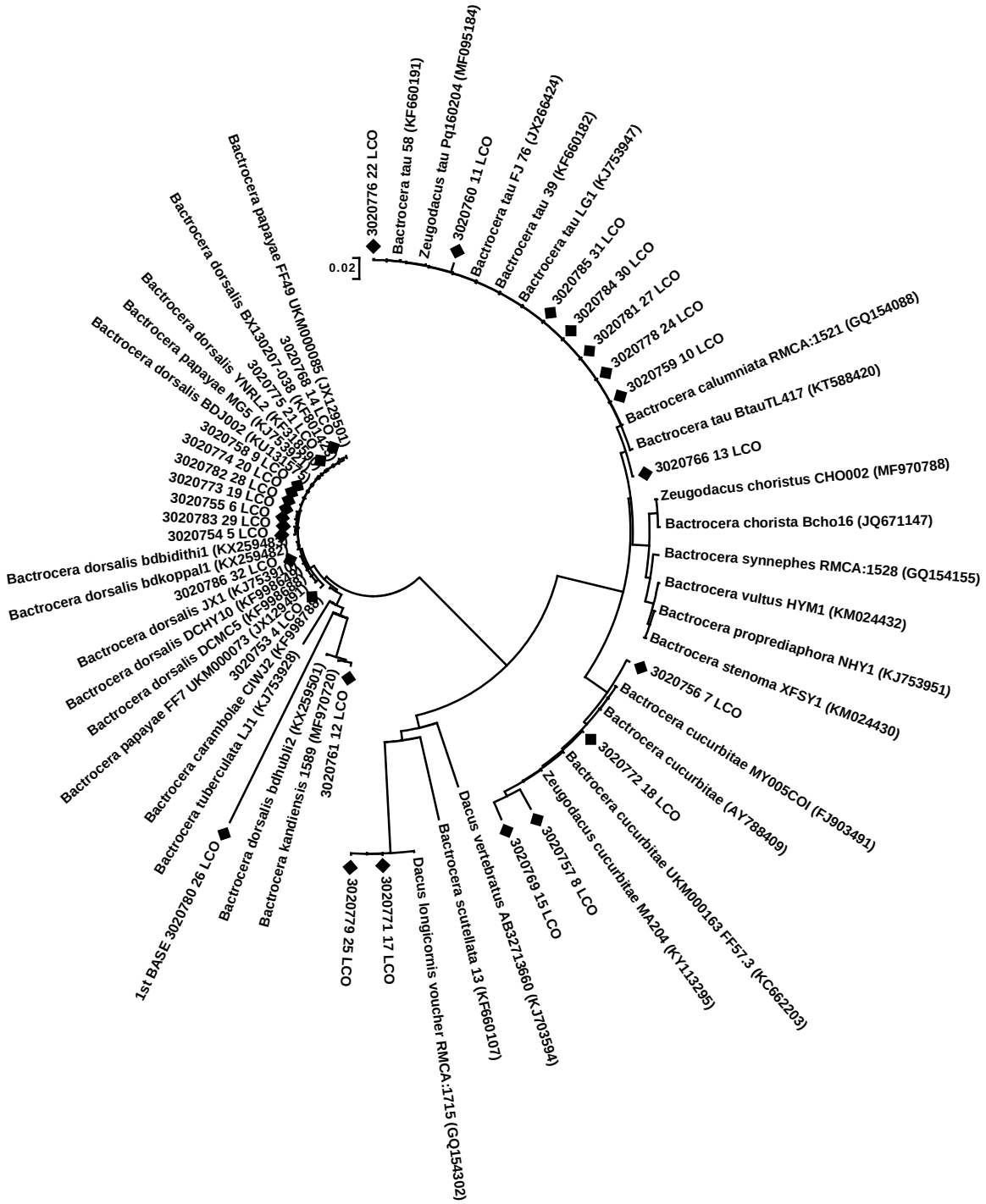



Fig. 2. Phylogenetic tree (circle) based on Cytochrome Oxidase I (COI) gene sequences revealing the phylogenetic relationships among the *Bactrocera* species. The 27 isolates from this study are indicated in the tree with a black diamond.

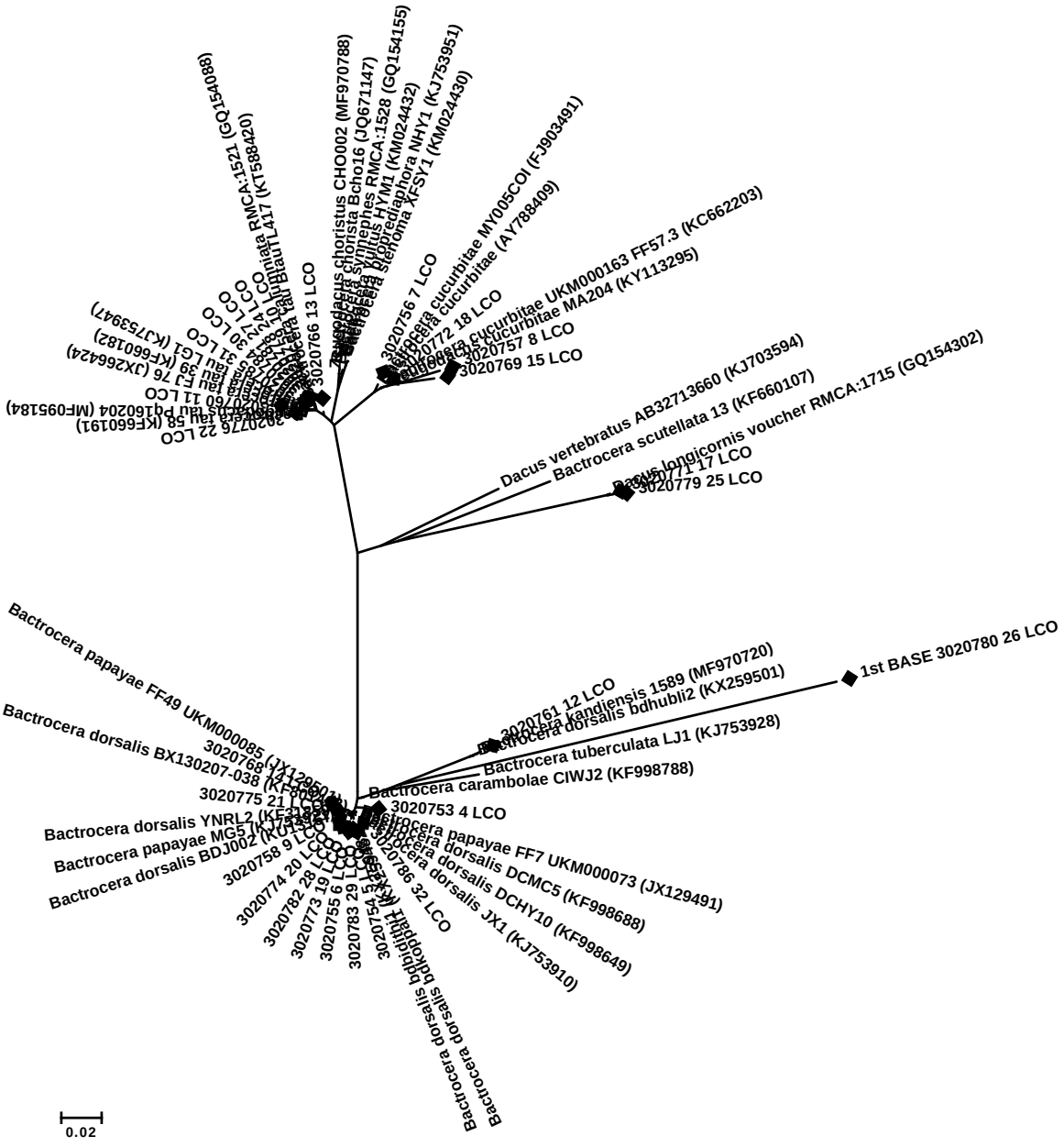



Fig. 3. Phylogenetic tree (radiant) based on Cytochrome Oxidase I (COI) gene sequences revealing the phylogenetic relationships among the *Bactrocera* species. The 27 isolates from this study are indicated in the tree with a black diamond.

**Results**

Molecular characterization of the 27 fruit fly isolates by COI sequencing indicated all the tested isolates were under the four major group of *Bactrocera* genus. Phylogenetic analysis of the isolates based on COI sequences revealed that there were four major species group viz. *Bactroera dorsalis* (13 isolates)*, B. tau* (8 isolates)*, B. cucurbitae* (4 isolates)*,* and *B. scutellata* (2 isolates). Among them three group viz. *Bactroera dorsalis, B. tau* and *B. cucurbitae* were most prevalent. *Bactroera dorsalis* group is distinct from *B. tau* and *B. cucurbitae* group (Fig. 1-3). Moreover, one fruit fly sample (LCO 11) collected from Barisal region supposed to be *Ceratitis capitata* by morphological characterization. But based on COI sequencing it was identified as *Bactroera tau.* Considering the significance of the species it will be re-sequenced for final conclusion.

**Distribution of the identified species:**

Distribution of the fruit fly species was assessed at different locations through pheromone trapping as well as month wise distribution was also done especially for three major species. It is observed from the Figure 1 that *Bactroera dorsalis* and *B. cucurbitae* are two major species of fruit fly and recorded from all the studied locations. Another major species *B. tau* was recorded from Gazipur, Jamalpur, Jeshore, Bogura and Khagrachari.

It is also revealed from Figure 2 that *Bactroera dorsalis* outnumbered all other species and can be considered as the most prevalent species of fruit flies in Bangladesh followed by *B. cucurbitae*. *B. tau* also recorded throughout the year; however their number is less than those two major species. The highest numbers of fruit flies, all three major species, were recorded during hot and humid seasons, viz. during the months of June and July. On the other hand lowest fruit fly populations were recorded during cool months, December-January.

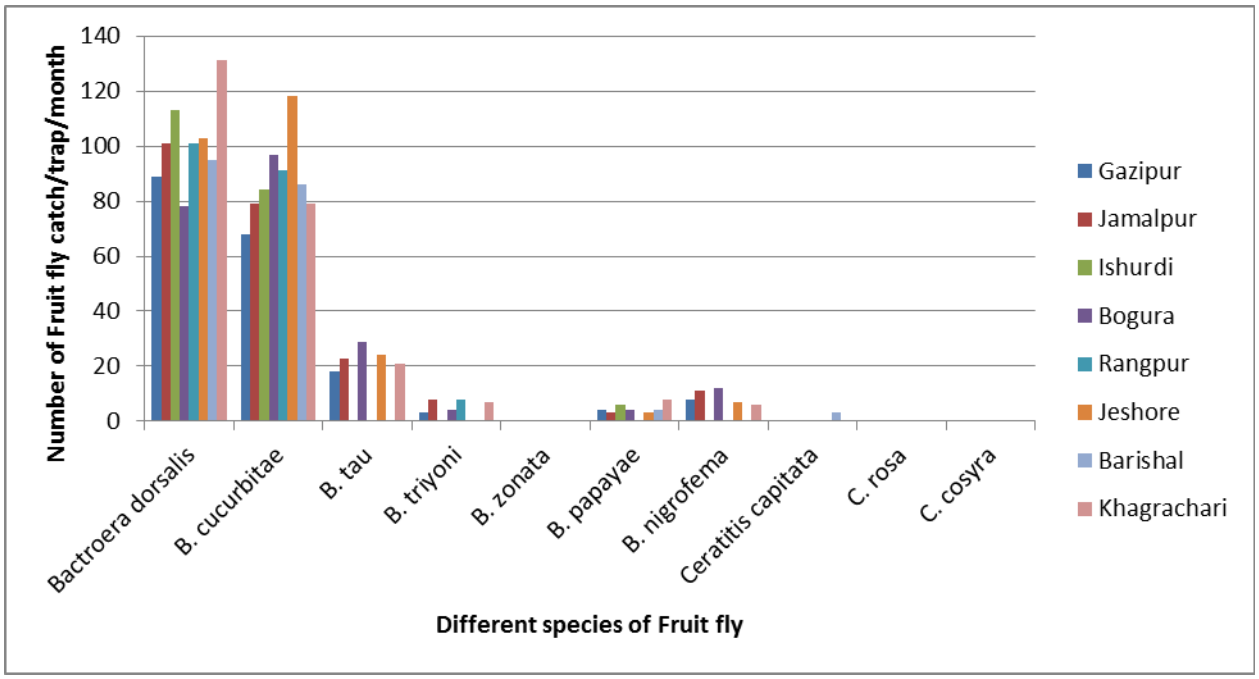



Figure 1: Distribution of different species of fruit fly at different locations

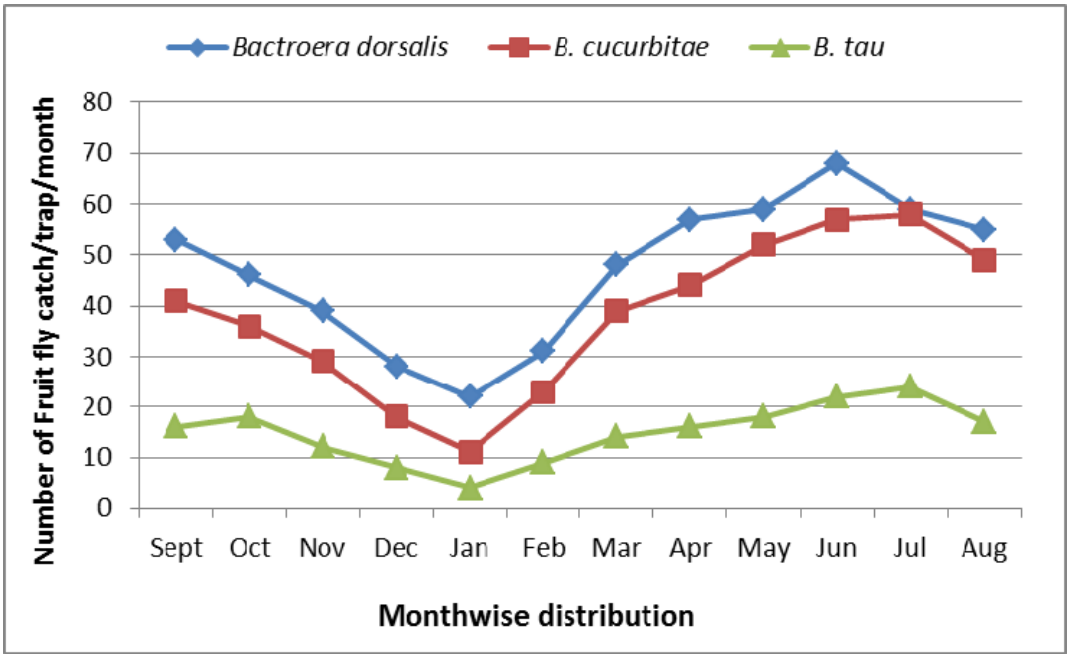


Figure 2: Month wise distribution of three major species of fruit fly at different locations

**Development of bio-rational based management packages against different species of fruit flies.**

**Efficacy of management options against fruit fly attacking different fruits**: One study was undertaken at Fruit Farm, BARI, Gazipur during 2017 cropping seasons to control fruit fly on guava. There were 05 treatments and 04 replications and the studies were arranged at scattered randomized complete block design. The treatments were assigned as follows: $T_1$= attract and kill method: setting of methyl euginol pheromone lures at the border plants at 10 m distance and female attractant in the inner rows at 15 m distance (one and half months before fruit harvesting), $T_2$ = setting of methyl euginol pheromone traps at 12 m distance from two months before fruit harvesting, $T_3$= bagging of fruits at around one and half months before fruit harvesting, $T_4$= Spraying of Cypermethrin 10EC @1ml/liter of water & $T_5$= Untreated control. Data of infested fruits were collected during fruit harvesting.

**Results and discussion:** It is revealed from the Table 1 that the significantly lowest infestation 0.00% and highest healthy fruit yield 20.28 ton/ha was recorded from the Treatment 3 comprising bagging with polythene, followed by Treatment 1 comprising sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) and Treatment 2, Sanitation+ Pheromone (culure) mass trapping in water trap. The highest infestation and lowest yield was recorded from untreated control plots followed by Cypermetrin 10 EC spraying.

Table 1. Fruit infestation and yield of **guava** during summer 2017 at Gazipur

| Treatments | Fruit infestation (%) | Yield (t/ha) |
|---|---|---|
| $T_1$=Sanitation+ Attract & Kill | 3.18 a | 18.52 a |
| $T_2$= Sanitation+ Pheromone | 15.36 b | 16.38 a |
| $T_3$= Polythene bagging | 0.00 a | 20.28 a |
| $T_4$= Spray Cypermethrin 10EC @1ml/l | 28.34 c | 11.38 b |
| $T_5$= Control | 37.33 d | 08.63 c |

**Efficacy of management options against fruit fly attacking different cucurbit fruits:** Two studies were undertaken, one on sweet gourd and another on bitter gourd at Entomology experimental field, BARI, Gazipur during 2017-18 cropping seasons to control fruit fly, *B. cucurbitae*.

**Sweet gourd:** efficacy of different management options of different species of fruit fly was studied on sweet gourd during winter 2017-18. There were 07 treatments with 03 replications. The treatments were $T_1$= Sanitation + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) + Soil recharge, $T_2$= Sanitation + attract & kill method, $T_3$= only attract & kill method; $T_4$= Sanitation+ Pheromone (culure) mass trapping in water trap+ Soil Recharge, $T_5$= Sanitation+ Pheromone mass trapping, $T_6$= Farmers Practice (Spraying of Cypermethrin 10EC @1ml/liter of water) and $T_7$ = untreated control. Data of infested fruits were collected during fruit harvesting. Economic analysis of different treatments for controlling fruit fly was also calculated.

**Results and discussion:** It is revealed from the Table 2 that the significantly the lowest infestation 3.17% and highest yield 22.70 tons/ha was recorded from the Treatment 1 comprising sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) + application of soil recharge (microbial pesticide to destroy pupa) followed by Sanitation + attract & kill method ($T_2$), $T_3$= only attract & kill method; $T_4$= Sanitation+ Pheromone (culure) mass trapping in water trap+ Soil Recharge, $T_5$= Sanitation+ Pheromone mass trapping. The highest infestation and lowest yield was recorded from untreated control plots. However the highest MBCR was calculated from the Sanitation + Pheromone Mass Trapping treatments (5.33) followed by sanitation + attract & kill method (4.42; Table 3). Lowest MBCR was calculated from the farmers' treatment.

Table 2. Fruit infestation and yield of bottle gourd during winter2017 at Gazipur

| Treatments | Fruit infestation (%) | Yield (t/ha) |
|---|---|---|
| $T_1$=Sanitation+ Attract & Kill+ Soil Recharge | 3.17 a | 22.70 a |
| $T_2$= Sanitation+ Attract & Kill | 6.18 ab | 21. 52 a |
| $T_3$= Attract & Kill | 9.76 b | 20.08 a |
| $T_4$= Sanitation+ Pheromone+ Soil Recharge | 9.39 b | 18.19 a |
| $T_5$= Sanitation+ Pheromone | 10.66 b | 17.02 a |
| $T_6$=Farmers Practice( Spray Cypermethrin 10EC @1ml/l | 15.58 c | 13.58 b |
| $T_7$= Control | 25.56 d | 10.69 c |

Table 3. Economic analysis after application of different treatments for controlling fruit fly of bottle gourd during winter 2017 at Gazipur

| Treatments | Yield (t/ha) | Pest management cost (Tk/ha) | Gross return (Tk/ha) | Net return(Tk/ha) | Adjusted net return(Tk/ha) | MBCR |
|---|---|---|---|---|---|---|
| $T_1$ | 22.7 | 20000/- | 181600/- | 162600/- | 77080/- | 3.85 |
| $T_2$ | 21. 52 | 16000/- | 172160/- | 156160/- | 70640/- | 4.42 |
| $T_3$ | 20.08 | 15000/- | 160640/- | 145640/- | 60120/- | 4.0 |
| $T_4$ | 18.19 | 12000/- | 145520/- | 133520/- | 48000/- | 4.0 |
| $T_5$ | 17.02 | 8000/- | 136160/- | 128160/- | 42640/- | 5.33 |
| $T_6$ | 12.58 | 7000/- | 100640/- | 93640/- | 8120/- | 1.16 |
| $T_7$ | 10.69 | - | 85520/- | - | - | - |

Soil Recharge: 800/-/kg, Attract & Kill: 75 Tk/kit, Sanitation: 2 labour/ha, Pheromone lure+trap:70/-/trap, Insecticide:150Tk/100ml, Farm gate price 20 Tk/ fruit

**Bitter gourd:** Efficacy of different management options of different species of fruit fly was studied on bitter gourd summer 2018. There were 04 treatments with 03 replications. The treatments were $T_1$= Sanitation+ Pheromone mass trapping, $T_2$= Sanitation + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing), $T_3$= Farmers Practice (Spraying of Cypermethrin 10EC @1ml/liter of water) and $T_4$ = untreated control. Data of infested fruits were collected during fruit harvesting. Economic analysis of different treatments for controlling fruit fly was also calculated.

**Results and discussion:** It is revealed from the Table 4 that the significantly the lowest infestation 5.35% and highest yield 18.34 tons/ha was recorded from the Treatment 2 comprising sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within

one month of seed sowing) followed by $T_4$= Sanitation+ Pheromone (culure) mass trapping in water trap. The highest infestation (31.61%) and lowest yield (8.90 t/ha) was recorded from untreated control plots. However the highest MBCR (Table 5) was calculated from the Sanitation + Pheromone Mass Trapping treatments (9.38) followed by sanitation + attract & kill method (8.44). The lowest MBCR was calculated from the farmer's treatment (2.32).

Table 4. Fruit infestation and yield of bitter gourd during summer 2018 at Gazipur

| Treatments | Fruit infestation (%) | Yield (t/ha) |
|---|---|---|
| $T_1$= Sanitation + Pheromone mass trapping with culure | 6.54 a | 16.82 a |
| $T_{2=}$ Sanitation + Pheromone mass trapping with Attract & Kill | 5.35 a | 18.34 a |
| $T_{3=}$ Farmers Practice( Spray Cypermethrin 10EC @1ml/l | 22.4 b | 10.45 b |
| $T_{4=}$ Control | 31.61 c | 6.90 c |

Table 5. Economic analysis after application of different treatments for controlling fruit fly of bitter gourd during summer 2018 at Gazipur

| Treatments | Yield (t/ha) | Pest management cost(Tk/ha) | Gross return (Tk/ha) | Net return(Tk/ha) | Adjusted net return(Tk/ha) | MBCR |
|---|---|---|---|---|---|---|
| $T_1$ | 15.82 | 10000/- | 237300/- | 227300/- | 93800/- | 9.38 |
| $T_2$ | 18.34 | 15000/- | 275100/- | 260100/- | 126600/- | 8.44 |
| $T_3$ | 10.45 | 7000/- | 156750/- | 149750/- | 16250/- | 2.32 |
| $T_4$ | 8.90 | - | 133500/- | - | - | |

Attract & Kill: 75Tk/kit, Sanitation: 2 labour/ha, Pheromone lure+trap:70 Tk./trap, Insecticide:150Tk/100ml, Farm gate price 15Tk/ kg

**On-farm validation of the developed management packages:**

On farm validation of the developed management packages was done on different fruit and cucurbit crops at Shreepur, Gazipur (stripped gourd), Bagarpara, Jeshore (bitter gourd) and on mango at Kallyanpur, Chapainawabgonj. Those trails were started since April 2018 and ended during August 2018. The results of the trials are as follows:

Location: Kallyanpur, Sadar, Chapainawabgonj. The trial was done on mango plants. There were two treatments, i) IPM: Sanitation + Pheromone mass trapping with Attract & Kill and ii) Farmers Practice: spray Cypermethrin 10EC @1ml/liter. It was observed that 84.68% fruit infestation reduction was recorded in the IPM trials resulted in 42.9% yield increase of healthy fruits.

Table 6. Field validation of the attract and kill based IPM technology for controlling fruit fly in mango during summer 2018 at Chapainawabgonj

| Treatments | Fruit infestation (%) | % infestation reduction over FP | Yield (t/ac) | % yield increase over FP |
|---|---|---|---|---|
| IPM = Sanitation + Pheromone mass trapping with Attract & Kill | 2.88 a | 84.68 | 26.2 a | 42.9 |
| Farmers Practice (FP) (Spray Cypermethrin 10EC @1ml/l | 18.8 b | - | 18.4 b | |

Location: Shreepur, Gazipur. The trial was done on stripped gourd. There were two treatments, i) Sanitation + Pheromone mass trapping with Attract & Kill and ii) Farmers Practice (Spray Cypermethrin 10EC @1ml/liter. It was observed that 84.68% fruit infestation reduction was recorded in the IPM trials resulted in 39.7% yield increase of healthy fruits (Table 6).

Table 7. Field validation of the attract and kill based IPM technology for controlling fruit fly species complex in stripped gourd during summer 2018 at Gazipur

| Treatments | Fruit infestation %) | % infestation reduction over FP | Yield (t/ha) | % yield increase over FP |
|---|---|---|---|---|
| IPM = Sanitation + Pheromone mass trapping with Attract & Kill | 4.34 a | 80.64 | 32.44 a | 39.7 |
| Farmers Practice (Spray Cypermethrin 10EC @1ml/l | 22.42 b | - | 23.22 b | - |

Location: Bagarpara, Jeshore. The trial was done on bitter gourd. There were two treatments, i) Sanitation + Pheromone mass trapping with Attract & Kill and ii) Farmers Practice (Spray Cypermethrin 10EC @1ml/liter. It was observed that 72.37% fruit infestation reduction was recorded in the IPM trials resulted in 52.54% yield increase of healthy fruits (Table 8).

Table 8. Field validation of the attract and kill based IPM technology for controlling fruit fly species complex in bitter gourd at Jeshore

| Treatments | Fruit infestation (%) | % infestation reduction over FP | Yield (t/ha) | % yield increase over FP |
|---|---|---|---|---|
| Sanitation + Pheromone mass trapping with Attract & Kill | 5.78 a | 72.37 | 38.32 a | 52.54 |
| $T_{3=}$ Farmers Practice( Spray Cypermethrin 10EC @1ml/l | 20.92 b | - | 25.12 b | - |

**Analysis of the information**:

Collection of fruit flies has been done by deploying sex pheromone traps of 10 species of fruit flies from 08 locations. From the morphometric identification of the collected specimen using taxonomic keys through morphological variation 07 (seven) species of Fruit Fly was identified. They are five species of genus *Bactrocera, B. cucurbitae, B. tau, B. triyoni, B. papaya, B. dorsalis, B. nigrofema* and one species of *Ceratitis, C. capitata.* However, *Ceratitis capitata,* also known as Mediterranean fruit fly (Medfly), which is one of the world's most destructive fruit pests. The fruit fly species were collected only from Rahmatpur, Barishal. The Mediterranean fruit fly attacks more than 260 different fruits, flowers, vegetables, and nuts. Thin-skinned, ripe succulent fruits are preferred by the pest. Several species of cucurbits have been recorded as hosts of the medfly.

However, molecular analysis through phylogenetic analysis of the isolates based on COI sequences revealed that there were four major species group viz. *Bactroera dorsalis* (13 isolates)*, B. tau* (8 isolates)*, B. cucurbitae* (4 isolates)*,* and *B. scutellata* (2 isolates). Among them three group viz. *Bactroera dorsalis, B. tau* and *B. cucurbitae* were most prevalent. *Bactroera dorsalis* group is distinct from *B. tau* and *B. cucurbitae* group. Moreover, one fruit fly sample (LCO 11) collected from Barisal region, which was supposed to be *Ceratitis capitata* by morphological characterization, but based on COI sequencing it was identified as *Bactroera tau.*

Efficacy of management options against fruit fly attacking different fruits and vegetables were tested at BARI, Gazipur. On guava it was revealed that significantly lowest infestation 0.00% and highest healthy fruit yield 20.28 ton/ha was recorded from the bagging of fruits with polythene, followed by sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) treatment and Sanitation+ Pheromone (culure) mass trapping in water trap treatment. The highest infestation and lowest yield was recorded from untreated control plots followed by Chemical pesticide spraying.

On bottle gourd it is revealed that significantly the lowest infestation 3.17% and highest yield 22.70 t/ha was recorded from sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) + application of soil recharge (microbial pesticide to destroy pupa) treatment followed by Sanitation + attract & kill method, only attract & kill method, Sanitation+ Pheromone (culure) mass trapping in water trap+ Soil Recharge, Sanitation+ Pheromone mass trapping. The highest infestation and lowest yield was recorded from untreated control plots. However the highest MBCR was obtained from the Sanitation + Pheromone Mass Trapping treatments (5.33) followed by sanitation + attract & kill method (4.42). Lowest MBCR was calculated from the farmer's treatment.

On bitter gourd it is revealed that significantly the lowest infestation 6.54% and highest yield 15.82 tons/ha was recorded from the Treatment comprising sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) followed by the treatment Sanitation+ Pheromone (culure) mass trapping in water trap, The highest infestation (31.61%) and lowest yield (8.90 t/ha) was recorded from untreated control plots. However the highest MBCR was calculated from the Sanitation + Pheromone Mass Trapping treatments (9.38) followed by sanitation + attract & kill method (8.44). Lowest MBCR was calculated from the farmer's treatment (2.32).

**Awareness building of farmers and extension personnel:**

Three field days were arranged at the trial areas of fruit fly management with attract and kill method. At Chapainawabgonj field day were arranged on mango during July 2018, at Shreepur, Gazipur on stripped gourd and at Gaidghat, Bagarpara, Jeshore on bitter gourd during September 2018. A total of 150 participants (scientists, extension personnel, farmers) were attended in those field days.

12. **Research highlight/findings** (Bullet point – max 10 nos.):

- By morphometric identification of the collected specimen (from 08 locations of the country) using taxonomic keys through morphological variation, 07 (seven) species of Fruit Fly were identified. They are six species of genus *Bactrocera, B. cucurbitae, B. tau, B. triyoni, B. papaya, B. dorsalis, B. nigrofema* and one species of *Ceratitis, C. capitata. Ceratitis capitata,* also known as Mediterranean fruit fly (Medfly) is one of the world's most destructive fruit pests.
- However, molecular analysis through phylogenetic analysis of the isolates based on COI sequences revealed that there were four major species group viz. *Bactroera dorsalis* (13 isolates)*, B. tau* (8 isolates)*, B. cucurbitae* (4 isolates)*,* and *B. scutellata* (2 isolates). Among them three group viz. *Bactroera dorsalis, B. tau* and *B. cucurbitae* were most prevalent. *Bactroera dorsalis* group is distinct from *B. tau* and *B. cucurbitae* group.
- Moreover, one fruit fly sample (LCO 11) collected from Barisal region, which was supposed to be *Ceratitis capitata* by morphological characterization, but based on COI sequencing it was identified as *Bactroera tau.*
- Among the fruit fly species, *Bactroera dorsalis* and *B. cucurbitae* are two major species and recorded from all studied locations. Another major species *B. tau* was recorded from Gazipur, Jamalpur, Jeshore, Bogura and Khagrachari.
- However, *Bactroera dorsalis* outnumbered all other species and can be considered as the most prevalent species of fruit flies in Bangladesh followed by *B. cucurbitae*. *B. tau* also recorded throughout the year; however their number is less than those two major species.

- The highest numbers of fruit flies were recorded during hot and humid seasons, viz. during the months of June and July. On the other hand lowest fruit fly populations were recorded during cool and dry months, viz. December-January.
- Significantly lowest infestation by fruit fly complex and highest healthy fruit yield of different fruits were recorded from the bagging of fruits with polythene, followed by sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance, within one month of seed sowing) treatment and Sanitation+ Pheromone (culure) mass trapping in water trap treatment.
- On the other hand significantly the lowest infestation of fruit fly and highest yield was recorded from sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance) followed by the treatment Sanitation+ Pheromone (culure) mass trapping in water trap in the cucurbit crops.
- However the highest MBCR was calculated from the Sanitation + Pheromone Mass Trapping treatments followed by sanitation + attract & kill method in the cucurbit crops. Lowest MBCR was calculated from the farmer's treatment.

**B. Implementation Position**

**1. Procurement: There was no provision of capital item procurement in the sub-project.**

| Description of equipment and capital items | PP Target | | Achievement | | Remarks |
|---|---|---|---|---|---|
| | Phy (#) | Fin (Tk) | Phy (#) | Fin (Tk) | |
| (a) Office equipment | | | | | N/A |
| (b) Lab &field equipment | | | | | N/A |
| (c) Other capital items | | | | | N/A |

**2. Establishment/renovation facilities: There was no provision of establishment/renovation in the sub-project.**

| Description of facilities | Newly established | | Upgraded/refurbished | | Remarks |
|---|---|---|---|---|---|
| | PP Target | Achievement | PP Target | Achievement | |
| | | | | | N/A |

**3. Field day organized:**

| Description | Number of participant | | | Duration (Days/weeks/ months) | Remarks |
|---|---|---|---|---|---|
| | Male | Female | Total | | |
| Field day | 115 | 35 | 150 | 1 day for each field day | 03 field days were arranged at Gazipur, Chapainawabgonj and Jeshore |

**C. Financial and physical progress**

**Fig in Tk**

| Items of expenditure/activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| A. Contractual staff salary | 2,78,530 | 2,63,530 | 2,63,530 | 0 | 100 | N/A |
| B. Field research/lab expenses and supplies | 12,88,470 | 12,19,840 | 12,19,840 | 0 | 100 | N/A |
| C. Operating expenses | 2,88,000 | 2,7,8017 | 2,78,017 | 0 | 100 | N/A |
| D. Vehicle hire and fuel, oil & maintenance | 2,70,000 | 2,38,519 | 2,38,519 | 0 | 100 | N/A |
| E. Training/workshop/seminar etc. | 1.20.000 | 90.000 | 90.000 | 0 | 100 | N/A |
| F. Publications and printing | 80.000 | 30.000 | 30.000 | 0 | 100 | N/A |
| G. Miscellaneous | 75.000 | 41.939 | 41.939 | 0 | 100 | N/A |
| H. Capital expenses | 0 | 0 | 0 | 0 | 100 | N/A |
| Grand total | **24,00,000** | **21,61,845** | **21,61,845** | 0 | | |

**D. Achievement of Sub-project by objectives: (Tangible form)**

| **Specific objectives of the sub-project** | **Major technical activities performed in respect of the set objectives** | **Output(i.e. product obtained, visible, measurable)** | **Outcome (short term effect of the research)** |
|---|---|---|---|
| Collection & identification of fruit flies | i) Collection and documentation of species composition of fruit flies<br>ii) Distribution of the identified species | i) By morphometric 07 (seven) species of FFly were identified.<br>ii) However, through molecular identification three major species *Bactroera dorsalis , B. tau, B. cucurbitae* were identified.<br>iii) *Bactroera dorsalis* outnumbered all other species and can be considered as the most prevalent species of fruit flies in Bangladesh. | Species complex of fruit fly is prevalent throughout the country. |
| Development of bio-rational based management options of different fruit fly species | Studies on the development of bio-rational management options on different fruits and vegetables. | The effective management options of fruit fly species complex is Sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of culure pheromone lures at the border areas at 10 m distance and | Effective and economic management option of species complex of fruit fly has been developed. |

| | | | |
|---|---|---|---|
| | | female attractant in the inner rows at 12 m distance) | |
| Field validation of the developed technologies | i) Field validation studies at three different locations and on different fruits and cucurbit vegetables undertaken.<br>ii) Field days arrangement | Attract and kill method, an effective and economic management option of species complex of fruit fly has been developed. | Effective and economic management option of species complex of ffly has been field validated and being used by the farmers. |

**E. Publication made under the Sub-project:**

| Publication | Number of publication | | Remarks (e.g. paper title, name of journal, conference name, etc.) |
|---|---|---|---|
| | Under preparation | Completed and published | |
| Technology booklet | | √ | "Effective management of fruit fly with attract and kill method" in bangla. "আর্কষন ও মেরে ফেলা পদ্ধতির মাধ্যমে বিভিন্ন প্রজাতির ফলের মাছি পোকার কার্যকরী ব্যবস্থাপনা" |
| Journal publications | √ | | 1. Species complex of fruit fly infesting different fruits and vegetables in Bangladesh. Journal: Bangladesh Journal of Entomology<br>2. Bio-rational management of fruit fly complex in different fruits and vegetables. Journal: Bangladesh Journal of Entomology |

**F. Technology/Knowledge generation/Policy Support (as applied):**

**i. Generation of technology (Non-commodity)**

Attract and kill method, an effective and economic management option of species complex of fruit fly has been developed, comprising of sanitation (destruction of infested fruits from the soil) + attract & kill method (setting of cuelure pheromone lures at the border areas at 10 m distance and female attractant in the inner rows at 12 m distance).

ii. **Generation of new knowledge that help in developing more technology in future**

Instead of one species, Fruit fly population of one location comprising of several species. However, through molecular identification three major species *Bactroera dorsalis , B. tau, B. cucurbitae* were identified. *B. dorsalis* outnumbered all other species and can be considered as the most prevalent species of fruit flies in Bangladesh.

**iii. Technology transferred that help increased agricultural productivity and farmers' income**

Attract and kill method, an effective and economic management option of species complex of fruit fly attacking various fruits and vegetables, which also considered as one of the most destructive pests. The inputs have already been registered and available in the market as a commercial product.

**iv. Policy Support**

As fruit fly infestation is not done only with one species, rather species complex is responsible for the fruit infestation and yield loss, so appropriate management option(s) should be undertaken addressing all the species. In that respects attract and kill method is an effective and economic management option. The inputs have already been registered and available in the market as a commercial product.

**G. Information regarding Desk and Field Monitoring**

**i) Field Monitoring (time& No. of visit, Team visit and output):**

Three field monitoring were done, i) Crop division, BARC: 01 visit, ii) PIU NATP II team: 01 visit, iii) BARI monitoring team: 01 visit.

**H. Lesson Learned/Challenges (if any): Not applicable**

**I. Challenges (if any): Not applicable**

Signature of the Principal Investigator
Date: 31 December 2018
Seal

Counter signature of the Head of the organization/authorized representative
Date: 31 December 2018
Seal