DEVELOPMENT OF ARTIFICIAL LARVAL DIET AND OPTIMIZATION OF STERILE MALE RATIO OF ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (HENDEL)

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CERTIFICATE

This is to certify that thesis entitled, **"DEVELOPMENT OF ARTIFICIAL LARVAL DIET AND OPTIMIZATION OF STERILE MALE RATIO OF ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (HENDEL)"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN ENTOMOLOGY**, embodies the result of a piece of bona fide research work carried out by **ASRIN ZAHAN**, **Registration No. 06-02060** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: December, 2012   **Prof. Dr. Md. Abdul Latif**

Dhaka, Bangladesh   **Supervisor**

Dedicated
To My

Beloved Parents

&

Teachers

ACKNOWLEDGEMENT

DEVELOPMENT OF ARTIFICIAL LARVAL DIET AND OPTIMIZATION OF STERILE MALE RATIO OF ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (HENDEL)

ABSTRACT

A study was conducted to develop artifitial larval diet and optimization of the sterile male ratio of oriental fruit flies, *Bactrocera dorsalis* (Hendel) for possible field application of Sterile Insect Technique (SIT). Population fluctuations of the fruit fly were monitored in Atomic Energy Research Establishment campus, Ganakbari, Savar, Dhaka from January 2012 to December 2012 using methyl eugenol baited Mcphail traps. High level of fruit fly population was found throughout the year in all the study areas except February. Mean monthly capture was highest in May. Different ratios between irradiated and unirradiated male of fruit flies were allowed to mate with a fixed number of unirradiated females of fruit flies in different cages to find out the rate of suppression against wild population. In case of efficiency of artifitial larval diet the highest hatching percentage and the lowest larval duration was observed from the 4.87-4.89 pH. But based on the highest number of pupae, percent flier, percent adult emergence and lowest pupal duration, diet having 5.22-5.26 pH was found to be economical and suitable for mass scale rearing of *B. dorsalis.* Radiosensitivity of pupae was found to be decreased as age increased. Highest pupal duration and no normal adults were found after imposing gamma radiation in 5-days old pupae treated with 60 Gyga dose. Radiation significantly increased the unemergence and decreased the normal emergence of fruit fly. The sterilizing doses were recorded as 60 Gy for 5-days old pupae. The 1:9 ratio of unirradiated male and irradiated male was found as the best for suppression of wild population of *B. dorsalis* in both laboratory and field trials.

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Introduction

CHAPTER I

INTRODUCTION

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) is a very destructive pest of fruit in areas where it occurs (White and Elson-Harris 1994). It is established in numerous areas of Bangladesh. The oriental fruit fly has been recorded from more than 150 kinds of fruits and vegetables including apricot, banana, mango, guava, papaya, avocado, citrus, fig, coffee, peach, pea, pineapple, and tomato (Koyama 1989).

Innumerable insect pests cause enormous economic losses to our agricultural products. Tephritid fruit flies (Diptera) are considered as an insect group of major economic significance. Several representatives of these flies (*Bactrocera, Ceratitis, Anastrepha* etc.) are known to attack different types of commercial and wild fruits and vegetables, causing considerable damage to agricultural crops. They constitute one of the largest and most diversified group of insects and consist of over 4000 species, of which nearly 700 belong to the Dacine fruit flies (Fletcher 1987). Nearly 250 species are of economic importance and are distributed widely in temperate, sub-tropical, and tropical regions of the world (Christenson and Foote 1960).

Bactrocera genus have more or less 440 species distributed principally in tropical Asia, the South Pacific, and Australia (White and Elson-Harris 1994). Flies in the genus *Bactrocera* are of particular concern throughout

the Asia and Australia, where they constitute a significant threat to agricultural resources (White 1996, Kinnear *et al.* 1998, Kim *et al*. 1999). Forty three species have been described under the genus *Bactrocera,* which are distributed throughout the temperate, tropical and sub-tropical regions of the world, especially Asia, Africa and Australia, but India is considered as its native home (Syed 1969, Cavalloro 1983, Drew and Hooper 1983, Munro 1984, Fletcher 1989). Eighty seven species of Tephritidae in India of which the genus, *Bactrocera* caused heavy damage to fruits and vegetables in Asia (Nagappan *et al.* 1971).

In Bangladesh six species of fruit flies under the family Tephritidae have been recognized. These are *B. dorsalis* (Hendel), *B. cucurbitae* (Coquillett), *B. tau* (Walker), *B. zonata* (Saunders), *B. diversus* (Coquillett) and *Dacus longicornis* (Wiedemann) (Wadud *et al*. 2005). Among them one of the most destructive insect is the oriental fruit fly, *Bactrocera dorsalis* (Hendel) which damages our fruits and vegetables in every year. This fruit fly is a very important group of pests for many countries due to their potential to cause damage in fruits, vegetables and to their potential to restrict access to international markets for plant products that can host fruit flies. Oriental fruit fly is so highly attracted to guava, and so effective at utilizing this host, that it has displaced Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) as the principal pest of guava, and significantly lowered the overall density of Mediterranean fruit fly in Hawaii.

Among vegetables, pepper, tomato, and watermelon are reportedly attacked. The high probability of introduction of oriental fruit flies associated with a wide range of hosts results in restrictions imposed by many importing countries to accept fruits from areas in which these pests are established. The adult flies feed on secretions of extrafloral nectaries, honeydew, rotting fruit, bird dung, and other liquefied items. The adults survive only three days without water, and six days with water, but no sources of carbohydrate (Harris *et al.* 1993). The ability of flies to disperse long distances to obtain food.

The classical approach of using of pesticides cannot be relied upon because of environmental and health hazards to both applicator and consumers. The Sterile Insect Technique (SIT) involves the suppression of insect population through the release of sterile insects rendered infertile by gamma radiation. The sterile insect technique (SIT) has proved to be a well-established method of controlling key pests of agricultural importance (Snow 1988 and Vagras 1989). The Sterile Insect Technique, best known by its acronym SIT and also identified as the Sterile Insect Release Method (SIRM), is a biologically-based method for the management of key insect pests of crops. The Sterile Insect Technique is defined as a method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species (Dowell and Siddiqui 2000). The sterile insect techniques is a method of biological control, whereby over

whelming numbers of sterile insects are released. The released insects are normally male. The sterile male compete with wild male for pairing with female insects. If a female mates with a sterile male then it will have no offspring, thus reducing the next generation population.

Sexual sterility can be induced in target pest species by means of chemical and physical agents including alkylating agents, antimetabolites, X-rays, gamma rays and neutrons. To date, no chemical sterilants have been discovered that can be used without at least presenting some hazard to workers in mass-rearing factories, nor are chemicals yet available that can be applied to the indigenous pest population without risk to non-target species. Consequently, in current practice, sexual sterility is induced with radiation emitted from radioisotopes such as caesium-137 and cobalt-60. The dosage of radiation applied must have no significant adverse effect on the males longevity, searching behavior and mating ability (Barry and Morse 2004). SIT is a species specific environmentally safer process and when applied it could eradicate the target pests successfully from a definite area. Sterile insects are not self-replicating and therefore cannot become established in the environment. SIT does not introduce exotic species into an ecosystem. SIT includes rearing millions of insect in the laboratory using artificial diet. In Bangladesh this fly is a serious pest in extensive mango growing areas (Khan *et al*. 2000). Researchers have established the laboratory rearing methods of this fly using different natural hosts. But for

SIT purposes that rearing method is not suitable enough to supply a huge amount of larvae/pupae within short period of time. Thus it is essential to develop cost effective artificial larval diet. In Bangladesh, A few research experiments have been done on the artificial larval diet development and sterile male ratio optimization of *B. dorsalis.*

Considering the above facts view in mind, the experiment has been undertaken with the following objectives:

1. To develop effective artificial larval diet for continuous supply a huge number of larvae/pupae in short period of time to success the SIT program.

2. To optimize the sterile male ratio of oriental fruit fly for suppression of *B. dorsalis* population.

Chapter 2
Review of literature

CHAPTER II

REVIEW OF LITERATURE

Oriental fruit fly, *Bactrocera dorsalis* is considered as economically most important pest because of their multivoltine life cycle with an explosive reproductive capacity. Control action by growers mainly dependent on chemical measures. SIT has been used successfully for the suppression, eradication, containment and prevention of some species of fruit flies in different countries (Balock *et al.* 1963).

2.1 General review of Oriental fruit fly

2.1.1 Nomenclature

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Tephritidae

Genus: *Bactrocera*

Species: *Bactrocera dorsalis*

2.1.2 Origin and distribution

Oriental fruit fly is well distributed over most of the Asian countries like Bangladesh, Bhutan, Cambodia, China (southern), Hong Kong, India (numerous states), Indonesia, Japan (Ryukyu Islands), Laos, Malaysia,

Myanmar, Nepal, Ogasawara Islands, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam. In North American region fruit fly is distributed over United States (Hawaii, with reoccurring infestations and eradications in California and Florida) and in Pacific Islands Mariana Islands, Tahiti (CABI 1994, Vargas *et al*. 2007). In the United States, it is currently present on all major Hawaiian Islands after being accidentally introduced into Hawaii in 1944 or 1945 (Barry *et al*. 2003).

Four major oriental fruit fly infestations in California were eradicated between 1960 and 1997. Additional infestations were detected in 2002 and 2004, and were eradicated in 2006 and 2007, respectively. In July 2010, fruit flies were discovered in traps in Sacramento and Placer counties. A quarantine was established and an eradication program begun (Anonymous 2010)**.**

While not established in Florida, oriental fruit flies are occasionally trapped in this state, recent occurrences being in 1999, 2000, 2001, 2002, 2007, 2008 and 2010, but easily eliminated (Steck 2007). India is considered its native home, and throughout most of Southeast Asia. Dhillon *et al*. (2005) reported that the Oriental fruit fly, *B. dorsalis* is distributed widely in temperate, tropical, and sub-tropical regions of the world.

2.1.3 Biology of Oriental fruit fly

Oriental fruit fly is a holometabolous insect. So, it has four stages to complete its life cycle viz., egg, larva, pupa and adult.

Egg

Oriental fruit fly eggs average about 1.17 mm long and 0.21 mm wide, which is slightly smaller than melon fly. The female may puncture fruit and deposit her eggs, or she may take advantage of cracks or other wounds, including the ovipositor punctures of other flies. Eggs may be deposited at a depth of 5-6 mm in soft fruit, whereas they may be very near the surface in hard fruit. The upper- and lower-developmental thresholds for eggs are estimated at 38^0C and 12^0C, respectively (Brower *et al*. 1989). The average time for egg hatching is 1.6 days (Vargas *et al*. 1984) but hatching may be extended up to 20 days in cold weather.

Larva

Oriental fruit fly larvae are typical in form of tephritid fruit flies, cylindrical and broad posteriorly and tapering to point at the anterior end. There are three instars; all are whitish in color. The first instar ranges in size from about 1.2 -2.3 mm, whereas the second ranges from 2.5-5.7 mm and third instar ranges from 7.0-11.0 mm. The upper- and lower-developmental thresholds for larvae are estimated at 34^0C and 11^0C, respectively (Calkins *et al*. 1987). Larval development generally requires about 7.8 days, though its development time can range from 6 to 35 days.

Pupa

Mature larvae leave infested fruit and enter the soil, usually at the base of affected trees, to pupate. The puparia are 3.8-5.2 mm long and vary in color from tan to brownish-yellow. Pupal development requires about 10.3 days.

Adult

The adult fruit fly has a yellow to orange abdomen marked with a black "T". The thorax is predominantly black but bears two yellow stripes laterally. Oriental fruit fly lacks cross bands on its wings, and therefore is easily differentiated from melon fly. The adult of *B. dorsalis*, which is noticeably larger than a house fly, has a body length of about 8.0 mm; the wing is about 7.3 mm in length and is mostly hyaline. After adults emerge, a period of 6-12 days normally elapses before oviposition can occur. Copulation persists for 2-12 h. Males expel pheromone in a visible form resembling smoke (Anwar *et al.* 1982), similar to pheromone production by melon fly. Mating occurs at dusk in aggregations called "leks". Mating normally occurs at 4-5 day intervals. The adults continue to produce eggs for about two months. The female oriental fruit fly is more fecund than the related tephritids melon fly and Mediterranean fruit fly, and she produces an average of over 1400 eggs per female during a life span of about 80 days (Vargas *et al.* 1984). The oviposition rate is reported to be about 130 eggs per day.

The ovipositor is very slender and sharply pointed. Keys for distinguishing all life stages of these species were provided by Bustos *et al*. (2004), White and Elson-Harris (1992), and Follett *et al*. (2004).

Oriental fruit fly can complete a generation in about 30 days. In tropical climates, many overlapping generations per year are reported. Fruit fly abundance typically coincides with availability of ripening fruit, though they tend to be most common in summer and autumn (Vargas *et al*. 1996).

2.1.4 Host range

Oriental fruit fly causes major damage especially the fruits such as mango, guava, apricot, banana, papaya, avocado, citrus, fig, coffee, peach, pea, pineapple, tomato etc. which also harm other plants (White and Elson-Harris 1992, Yang *et al*. 1994). Doharey (1983) reported that it infests over 150 host plants, amongst which, fruits of *Aegle marmelos* (golden apple), *Anacardium occidentale* (cashew nut), *Annona reticulata* (bullock's heart), *Annona squamosa* (sugarapple), *Areca catechu* (betelnut palm), *Artocarpus altilis* (breadfruit), *Artocarpus heterophyllus* (jackfruit), *Averrhoa carambola* (carambola), *Capsicum annuum* (bell pepper), *Carica papaya* (papaw), *Chrysophyllum cainito* (caimito), *Citrus*, *Citrus aurantiifolia* (lime), *Citrus maxima* (pummelo), *Citrus reticulata* (mandarin), *Coffea arabica* (arabica coffee), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Dimocarpus longan* (longan tree), *Diospyros kaki* (persimmon), *Ficus racemosa* (cluster tree), *Flacourtia indica*, *Malpighia glabra*

(acerola), *Malus domestica* (apple), *Mangifera foetida* (bachang), *Mangifera indica* (mango), *Manilkara zapota* (sapodilla), *Mimusops elengi* (spanish cherry), *Momordica charantia* (bitter gourd), *Muntingia calabura* (Jamaica cherry), *Musa* (banana), *Nephelium lappaceum* (rambutan), *Persea americana* (avocado), *Prunus armeniaca* (apricot), *Prunus avium* (sweet cherry), *Prunus cerasus* (sour cherry), *Prunus domestica* (plum), *Prunus mume* (Japanese apricot tree), *Prunus persica* (peach), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Pyrus communis* (European pear), *Spondias purpurea*, *Syzygium aqueum* (watery rose-apple), *Syzygium aromaticum* (clove), *Syzygium cumini* (black plum), *Syzygium jambos* (rose apple), *Syzygium malaccense* (malay-apple), *Syzygium samarangense* (water apple), *Terminalia catappa* (Singapore almond), *Ziziphus jujuba* (common jujube), *Ziziphus mauritiana* (jujube) are the most preferred hosts (Allwood *et al*. 1999, Koyama 1989).

2.1.5 Nature and extent of damage

The damage to crops caused by oriental fruit flies result from oviposition in fruit and soft tissues of vegetative parts of hosts, feeding by the larvae and decomposition of plant tissue by invading secondary microorganisms.

These flies remain active throughout the year on one or the other hosts. During the severe winter months, they conceal and crowd together under dried leaves of bushes and trees. In the hot and dry season, the flies take shelter under humid and shady places and feed on honeydew of aphids

infesting the fruit trees (Dhillon *et al.* 2005). Generally, the females of this fly prefer to lay the eggs in soft tender fruit tissues by piercing them with their ovipositor. A watery fluid oozes from the puncture, which becomes slightly concave with leaching of fluid, and transforms into a brown resinous deposit (Gupta *et al.* 1978). After egg hatching, the larvae bore into the pulp tissue and make the feeding galleries. The fruit subsequently rotten or becomes distorted (Plate 2). Young larvae at the necrotic region and move to healthy tissue, where they often introduce various pathogens and hasten fruit decomposition (Arthur *et al*. 1989). Sometimes pseudo-punctures (punctures without eggs) have also been observed on the fruit skin (Bhatti 1970). This reduces the market value of the produce. The full-grown larvae come out of the fruit by making one or two exit holes for pupation in the soil. The larvae pupate in the soil at a depth of 0.5 to 15 cm. The depth up to which the larvae move in the soil for pupation, and survival depend on soil texture and moisture (Jackson *et al.* 1998; Pandey and Misra 1999).

Larval feeding damage in fruits is the most damaging (Wadud *et al.* 2005). Mature attacked fruits develop a water soaked appearance (Calcagno *et al*. 2002). Young fruits become distorted and usually drop. The larval tunnels provide entry points for bacteria and fungi that cause the fruit to rot (Collins *et al*. 2009). These maggots also attack young seedlings, succulent tap roots , stems and buds of host plants such as mango, guava, cucumber, custard apple and others (Weldon *et al*. 2008).

2.2 Seasonal occurrences

The successful application of the SIT requires knowledge of the target population's ecology, including estimates of the absolute density of the adult population, and how that density changes over time (Lindquist 1969, Lindquist *et al.* 1974, Knipling 1979, Ito and Yamamura 2005). Subsequent models, which contain parameters that incorporate behavioral and ecological information, provide more realistic estimates of over flooding ratios needed for desired levels of suppression (Knipling 1968, Barclay 2005, Klassen 2005). In practice, when high rates of increase are involved, the required over flooding ratios can be quite high. Population monitored by the artificial sex pheromone methyl eugenol baited traps. methyl eugenol [4allyl-1,2-dimethoxybenzene-carboxylate] are highly attractive kairomone lures to *B. dorsalis*.). Males of *B. dorsalis* including at least 58 species of Dacine are attracted to methyl eugenol (Metcalf 1998). Of the 46 *Dacus* species that are agricultural pests, 8 respond to methyl eugenol (Metcalf and Metcalf, 1992). Use of methyl eugenol and cue-lure mixtures has been reported in Taiwan and Okinawa to *B. dorsalis* and *B. cucurbitae* (Ito *et al.* 1976, Liu and Lin 1993). In the event that attractiveness was not reduced, using mixtures in control programs in Hawaii may reduce the amount of pesticide placed in the environment and the cost for treatment of both *B. dorsalis* and *B. cucurbitae.* Pheromone traps provide an easy and efficient method to monitor the abundance of fruit fly populations (Alyokhun *et al.*

2001). To monitor the fruit fly population pheromone trappings have been successfully used in different countries (Marwat and Baloch 1986, Gillani *et al.* 2002). Pheromone traps attract only male fruit flies but this could be used as indicators of the total population. Pheromones are also increasingly efficient at low population densities, they do not adversely affect natural enemies, and they can, therefore, bring about a long-term reduction in insect populations that cannot be accomplished with conventional insecticides (Toledo *et al.* 2010).

2.3 Sterile Insect Technique

The Sterile Insect Technique, best known by its acronym SIT and also identified as the Sterile Insect Release Method (SIRM), is a biologically-based method for the management of key insect pests of agriculture. The Sterile Insect Technique is defined as "*a method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species*" (Dowell and Siddiqui 2000). It is therefore a type of "birth control" in which wild female insects of the pest population do not reproduce when they are inseminated by released, radiation-sterilized males. In this type of autocidal control, sequential releases of the sterilized insects in adequate to wild male over flooding ratio's lead to a reduction in pest population numbers. Effective control using sterile insects is achieved when part of area-wide integrated pest management (AW-IPM) programme is established.

In its application, the target pest species is mass-reared, sexually sterilized and distributed over the range of the pest population being SIT does not introduce exotic species into an ecosystem (Gomez *et al*. 2007).

Sexual sterility can be induced in target pest species by means of chemical and physical agents including alkylating agents, antimetabolites, X-rays, gamma rays and neutrons (Makhmoor *et al.* 1998). To date, no chemical sterilants have been discovered that can be used without at least presenting some hazard to workers in mass-rearing factories, nor are chemicals yet available that can be applied to the indigenous pest population without risk to non-target species. Consequently, in current practice, sexual sterility is induced with radiation emitted from radioisotopes such as caesium-137 and cobalt-60. The dosage of radiation applied must have no significant adverse effect on the males' longevity, searching behaviour and mating ability (Hooper *et al.* 1971).

Sterile insect technique is one of the most wide applicable component of IPM which is a biologically-based genetic control method and has many advantages i.e., it is non-intrusive to the environment; does not harmfully affect non-target organisms; acts inversely density dependent and can be integrated with other biological control methods. Use of SIT provides an environmental safe and species-specific method to suppress or eradicate tephritid fruit flies of agricultural importance worldwide (Teal *et al.* 2007). In SIT, a large number of mass-reared insects are sterilized usually by

irradiation, and released into an area inhabited by a wild pest population, where the sterile males sexually compete with the wild males for copulation with wild females (Hallman 2000). As a result, the numbers of offspring in the next generation significantly decreased than it would be without the release of sterilized flies. The ova of wild females that are fertilized by sperm of sterile males fail to develop, reducing pest abundance in the next generation. (Taylor *et al.* 2001). Repeated releases of sterile males for several generations may eradicate the wild population (Knipling 1965, Bottrell 1979, Gilmore 1989 and Krafsur 1998). This technique (SIT) was conceived in the 1930 (Knipling 1965) and first applied on a significant scale in the 1950 against the New World screwworm *Cochliomyia hominivorax* (Coquerel) (Baumhover *et al.* 1955, Knipling 1968) and subsequently to a number of other pest species (Dyck *et al.* 2005). This technique has been used successfully against a number of pest species such as Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Gilmore 1989, Penrose 1996, Rossler *et al*. 2000), melon fly, *B. cucurbitae* (Kuba *et al*. 1996), Queensland fly, *Bactrocera tryoni* (Froggatt) (Fisher 1996), codling moth, *Cydia pomonella* (L.) and tsetse fly, *Glossina austeni* Newstead (Tan 2000, Wyss 2000, Hendrichs *et al.* 2005 and Klassen 2005).

2.4 Pupal radiosensitivity

Ionizing radiation breaks chemical bonds within DNA and other molecules, thereby disrupting normal cellular functions. Many tissues and functions of the insect may be disrupted by exposure to radiation (Vinson *et al.* 1969, Nation and Burditt 1994). Insects and other living organisms are able to repair molecular damage done by small amounts of ionizing energy (Alpen 1998), but large amounts are fatal or cause permanent sterility, and this is the basis for using irradiation to control insects in commodities. Radiotolerance can vary among the life stages of an insect, and between insect taxa. For example, Lepidoptera tend to be more radiotolerant than Diptera, Coleoptera, and Hemiptera, although there is considerable variation among the species that have been tested within these groups (Bakri *et al.* 2005). For individual species, radiotolerance normally increases with increasing developmental stage when the goal is to prevent successful reproduction (Follett 2008). Ionizing radiation is the method of choice for inducing reproductive sterility. Radiation can make an insect reproductively sterile by damaging the chromosomes of gonial cells, specifically causing germ-cell chromosome fragmentation that leads to the production of unbalanced gametes and subsequently the inhibition of mitosis and death of fertilized eggs or embryos (Robinson 2002, Klassen 2005). The use of ionizing radiation to induce mutations has provided a ready source of variation that has led to some of the pioneering development in animal

genetics (Atkinson *et al.* 2007). At first Bushland and Hopkins (1951) and later Knipling (1955) proposed the use of ionizing radiation to sterilize insect pests. The sterilization process is important in determining the quality of the released insects and their ability to compete with the wild population. Thus, optimization of the sterilization process is critical for the efficacy of SIT programmes and should be given due consideration. The absorbed dose of radiation that is used to induce sterility is of critical importance to a SIT program. Insects that receive too low an absorbed dose are not sufficiently sterile and those that receive too high an absorbed dose may be uncompetitive, reducing the effectiveness of the programme by requiring that a greater number of sterile insects must be released (Robinson 2002, Calkins and Parker 2005, Lance and McInnis 2005). Quite often, full (100%) sterility may not be the most favourable condition for a programme, and thus process optimization is necessary to balance sterility level and competitiveness, taking into consideration the factors that could affect the radiation sensitivity of insects and programme requirements. So research is essential to establish the relationship of dose to the level of sterility and competitiveness in the treated insects, and that a standardized dosimetry system and recognized dosimetry procedures are used (ISO/ ASTM 2005).

The radiation absorbed dose is expressed in System International Unites (SIU) units as gray (Gy) (1 Gy = 100 rad), where 1 Gy is equivalent to 1 joule (J) of absorbed energy in 1 kg of a specified material (1 Gy = 1 J/kg).

Currently, the most commonly used radiation for the SIT is gamma radiation from the radioisotopes Cobalt-60 (Co^{60}) and Caesium-137 (Cs^{137}). Radiosensitivity is the relative susceptibility of cells, tissues, organs or organisms to the harmful effect of <u>ionizing radiation</u>. Quality control is important for monitoring the performance of mass reared insects for use in the sterile insect technique. As a part of routine quality control tests the effect of irradiation must also be assessed and threshold values for each quality control parameter need to be established (Resilva *et al*. 2007).

2.5 Sterile male ratio

The most important factor in release strategies for SIT is the ratio of sterile to target wild insects (S:W ratio). The strategy could fail if the dispersion of sterile insects is such that there are patches of habitat where either there are no sterile flies or not enough of them to achieve a sufficiently effective (S: W ratio) should target wild flies be present as well. If a trap catches no sterile flies, the density of the latter in its vicinity is almost certain to be ineffective, especially if one or more target flies is trapped at the same time (Koyama *et al*. 2004). The most critical component of the SIT is effective mating (Teal *et al.* 2007). Therefore mating studies were conducted using fertile wild/mass reared female and sterile male to compare the competitiveness. The efficiency of mating between sterile males and wild females can be lost partially or entirely if the females also mate with wild males, and preferentially use sperm from the latter for fertilization (Lance *et*

al. 2000). To determine if the SIT is appropriate for use against an insect species, Knipling (1965) proposed that female must normally mate only once. This statement is still infrequently uttered strictly, although polyandry does not negate the basic principles of the technique. Indeed, the overall sterility induced into a population of ten females by a sterile: wild over flooding ratio of 9:1 should be the same whether each female mates once-nine with a sterile male and one with a wild male or each female mates ten times -nine times with sterile males and once with a wild male (Barclay 2005, Whitten and Mahon 2005).

2.6 Larval diet

Production of low cost but high quality sterile insecst is goal for successful sterile insect technique programmes (Nahar *et al.* 2006).Yeast products are the main nutritional component in the diet used to mass reared the adults and larvae of fruit flies in this program. (Rohlfs and Hoffmeister 2005). Laboratory colonization and mass production of fruit flies on artifitial diet may require several generations for the insects to adapt the artificial diet (Chan *et al.* 1998). If the liquid diet is incorporated into a mass rearing facility the cost associated with the disposal of the standard diet can be reduced and it saves potential space and lower environmental impact (Ronald and Kessing 2007).

Chapter 3

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The present study was conducted to establish the Sterile Insect Technique (SIT) method of controlling *Bactrocera dorsalis* (Hendel) in the laboratory and experimental field of Insect Biotechnology Division (IBD), Institute of Food and Rediation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka during January 2012 to February 2013. The materials and methods adopted in the study are discussed under the following heading and sub-headings.

3.1 Development of new larval diet

3.1.1 Diet preparation

The ingredients of diet were measured and mixed in a blender thoroughly. After mixing one litre diet was poured on stainless steel tray (6" wide×12" length). Four different compositions were prepared using different amount of HCl.

Table 1. Ingredients of larval artifitial diets of oriental fruit fly

Ingredients	Amount(g/ml)
Wheat bran	75g
Rice bran	50g
Soya bran	100g
Sweet potato	250g
Ascorbic acid	5g
Sugar	50g
HCl	2,3,4 and 0 ml
Sodium benzoate	4g
water	464,463,462 and 466 ml
Total	**1000ml**

3.1.2 Egg seedling

One litre diet was poured on each tray. An amount of 800-900(0.5 ml) were placed on a moist tissue paper placed on the diet. The eggs were found to hatch within 30 to36 hours.

3.1.3 Pupa collection

The trays were kept on saw dust in big bowls covered with clothes. After six days of seedling, the sawdust was sieved and pupae were collected. The pupae were kept on the large Petri dishes in small rearing cages (Plate 9) for adult emergence.

Plate 1. Small rearing cages

3.1.4 Temperature and humidity

The temperature and the relative humidity of the larval rearing room were maintained 28 ± 2^0C and $80\pm10\%$ respectively.

3.1.5 Flight ability test

Two days prior to adult eclosion, 100 pupae were placed in a petri dish (90 mm diameter) kept in a fruit fly rearing cage and a 10 cm PVC tube coated with talcum powder kept on the petri dish (Plate 10). The flies were allowed to emerge freely. Flight ability (flies escaping from the PVC tube) was determined based on the number of unemerged pupae and residual flies remaining in the petri dish. Then, the percentage of flying flies was calculated for each replicate. Three replications of 100 pupae from each group were performed.

Plate 2. PVC tube used for Flight ability test

3.1.6 PH test

PH level of four different compositions were tested using a pH meter made by Bangladesh Atomic Energy Commission.

3.2 Adult rearing

Rearing of adult oriental fruit flies (Plate 1a) were maintained in the laboratory of Insect Biotechnology Division (IBD) of IFRB, AERE, Savar, Dhaka (Plate 3a and 3b). Population fluctuations of the oriental fruit fly also monitored in AERE campus, Ganakbari, Savar, Dhaka. Mango, Guava, Custard Apple are grown in this area as small scale. Oriental fruit fly was monitored from January 2012 to December 2012 using methyl eugenol baited Mcphail traps (Plate 1b). Total eight Mcphail traps were set at approximately 100 m distances in each field and inspected every fortnightly.

Abiotic factors including air temperature, relative humidity, rainfall were analyzed with respect to the population fluctuation. This strain has been cultured for more than 30 generations using the artificial diet. About 5,000 adult flies were maintained in steel framed cages ($76 \times 66 \times 76$ cm) covered with wired net (Plate 3c and 3d). The front side of the cage had one hole covered with nylon mesh net to insert food, water and egging receptacles. The flies were supplied with protein based artificial diets *viz*., (i) baking yeast: sugar: water at 1:3: 4 ratio, and (ii) casein: yeast extract: sugar at 1:1:2 ratio. Water was supplied in a conical flask socked with cotton ball. The temperature and the relative humidity (RH) of the rearing room were maintained at 27 ± 2^{0}C and $75 \pm 5\%$ respectively, by using air conditioner (Model No. Movincool Classic Plus 26, USA) and a photoperiod of L14-D10, with photo phase starting at 0600h.

(a) Adult fruit fly   **(b) Mcphail trap**

Plate 3. Oriental fruit fly *Bactrocera dorsalis* and Mcphail trap used for trapping   oriental fruit fly

Plate 4. Fruit fly infested mango

(a)   (b)

(c)   (d)

Plate 5. Insect Biotechnology Division (IBD) laboratory (a and b) and steel framed cages for rearing of adult oriental fruit fly (c and d)

3. 3 Oviposition media

To collect huge number of eggs, the matured flies in the cage were provided with artificial egging receptacle for oviposition. A yellow plastic cylinder (10.5 × 20.5 cm) perforated with 400 oviposition holes was used as egging receptacle (Plate 4a). Crushed paste of banana was placed inside wall of the receptacle in order to stimulate oviposition. Adult flies laid eggs at the age of 13-14 days after emergence. Each female fly penetrate her ovipositor into the hole of the device and laid eggs in cluster (Plate 4b). The egging receptacle was enclosed with a wet sponge to prevent desiccation of eggs. Eggs collections were done 24 hours from 8:00 am to 8:00 am of the following day. Eggs that deposited in the egging receptacle were collected and washed with distilled water and sieved with a very fine screen and then measured volumetrically.

(a) **Perforated plastic cylinder used as egg receptacle**   (b) **Inside of egg receptacle**

Plate 6. Artificial egging devices for oviposition by fruit fly (a and b)

3.4 Larval rearing

Larvae of oriental fruit fly were reared in the laboratory using artificial standard larval diet. Each 3 kg of larval diet was composed of:

Components	Amount (g/ml)
Soya bran	400
Wheat bran	400
Sweet gourd	600
Suger	240
Vitamin C	10
Baking yeast	50
Sodium benzoate	4
Sorbic acid	2
Sweet potato	400
Ciric acid	2
Water	892
Total	3000

These components mixed together using a mechanical diet mixer. The pH of the diet was maintained at 4.5. Total 150 ml artificial larval diet was placed into a plastic larval tray (Plate 5) where 0.5 mg eggs (containing 800-900 eggs) were seeded on tissue paper on top of the diet media. The stackable

tray cultures were arranged on wooden rack, and then covered with nylon cloth to maintain high relative humidity necessary to stimulate egg hatching.

Plate 7. Plastic larval tray with modified liquid larval diet for larval rearing

3.5 Pupation media

Sawdust was used as pupation media. Larger wood particles were removed before the dusts were taken in the pupation jar. The larger bowl contained 1.5 to 4 cm thick sawdust (Plate 6). At the end of the developing period mature larvae leave the larval media after 7 days by popping-out of the trays into the saw dust used as the pupation media. The popping larvae then pupate into the sawdust. The pupae were collected regularly from sawdust by sieving with steel net. The pupae (pre-pupae) were placed again in the sawdust to avoid desiccation. The collected pupae were transferred into the adult rearing cages.

Pate 8. The larger plastic bowl as Pupation media contained saw dust

3.6 Irradiation

Pupae of different batches were irradiated by exposing them to gamma radiation from a radioactive Cobalt-60 source located at IFRB, AERE, Savar, Dhaka (Plate 7a and 7b). For pupal radiosensitivity 5 and 6 days old pupae of different batches were irradiated by exposing them to gamma radiation. To optimize the radiation dose of sterilization several batches of pupae were irradiated at 30, 40, 50 and 60 Gyga dose.

 (a) Control Panel of Gamma Irradiator   (b) Inside the Irradiation room

Plate 9. Radiation source used for sterilization of oriental fruit fly

3.7 Determination of Sterility Dose

To determine the sterilizing dose, virgin untreated females were allowed to mate with the irradiated males. In each cage, 300 irradiated males and 300 fertile virgin female were kept in a cage for mating. After 4 days of mating small pieces of banana were placed in each cage as an oviposition medium. The piece of banana was removed after 24 hours from the adult cage and placed in a small plastic bowl with sawdust for further larval development. After subsequent days the total number of pupa and pupal duration were counted and recorded.

3.8 Quality Control Tests Irradiated Flies

3.8.1 Percent of emergence

Before adult emergence, 100 pupae were placed in a Petri dish and allowed to emerge freely in a small screened cage. After completion of emergence, fully emerged, unemerged and deformed flies were counted and the percentage of emerging flies was calculated for each replication. Ten replications of 100 pupae from each group (originating from different emergence dates) were performed.

3.9 Optimization of Sterile Male Ratio

The male adults of *B. dorsalis* emerged from the pupae of 5 days old irradiated with 60Gy gamma radiation were segregated into separate cage. These males were allowed to mate with fertile virgin females at 1:1:0; 1:1:2; 1:1:5 and 1:1:9 (Normal female: Normal male: Irradiated male) ratios in separate adult cages.

- One cage was arranged with 60 untreated female were allowed to mate with 60 untreated male from control batch.

- 60 untreated female and 20 untreated male from control batch were allowed to mate with 40 irradiated male at 1:2 ratio.

- 60 untreated female and 10 untreated male from control batch were allowed to mate with 50 irradiated male at 1:5 ratio.

- 60 untreated female and 6 untreated male from control batch were allowed to mate with 54 irradiated male at 1:9 ratio and

Each ratio was replicated three times and in each replication 120 flies were used. Ratio between irradiated males and unirradiated males with unirradiated females was optimized by recording the number of pupal recovery, normal adult emergence, deformed flies emergence and unemergence percentage and compared with each other.

3.10 Suppression experiments

The adult emerged from the irradiated (50 Gy) pupae were sexed. The males were allowed to mate 1:2, 1:5 and 1:9 (normal male: irradiated male) ratios with virgin females in separate adult cages. A piece of banana (200 gm) as oviposition media was placed in the cage 4th day after mating. The banana was removed after 24 hrs and placed on artificial diet. The total no of pupae and adult emergence were counted and recorded. In field cage trials, 9 days old 300 virgin females with corresponding number of irradiated and unirradiated males were released in the netted area (25×10×12.5cm) in the natural environment (plate 8). Rest of the experimental procedure is similar as described above. Control batches were maintained for both the experiment. Data was subjected to two-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) for evaluating ratio-wise difference.

Plate 10. Suppression experiments in field cages

3.11 Assessment of seasonal occurrences

The field survey of *B. dorsalis* was carried out in a vegetable field *viz.,* Ganakbari, Savar, Dhaka ($23^0 59''$N, $90^0 16''$E) during January 2012 to december 2012. Ganakbari, Savar is located at northwest side of Dhaka, the capital of Bangladesh. It is a high plain land with red hilly soil. Many types of vegetables are cultivated throughout the year in this area. Eight McPhail traps were set at approximately 100 m distances in the field and inspected every fortnightly. Each trap was placed on bamboo poles or on tree brance at a height of 1m above the ground. A parapheromone lure (methyl eugenol) stick was suspended inside each trap, near the center. The parapheromomne lure stick was consisted of a small cotton rope impregnated with 2 ml of lure [4allyl-1,2-dimethoxybenzene-carboxylate] marketed by Safe Agriculture Bangladesh Ltd. A cotton ball soaked with 100 ml sevin-solution (insecticide, Bayer Bangladesh) was placed inside each trap as insect killer, which was replaced at 15 days intervals throughout the year. Male flies were attracted by the lures, and were quickly killed by the insecticide on the cotton ball. The flies of *B. dorsalis* species were identified and counted in each trap at 15 days intervals. Trap capture rates were calculated based on four traps at each site. The monthly meteorological data used in the present study provided by the Center of Meteorological Department, Dhaka, Bangladesh.

The trapped flies were removed from the traps and counted after every fortnight. One-way Analysis of Variance (ANOVA) was used for month-wise differences in the fruit fly population and for the difference in the population at different experimental sites. Treatment means were separated by Duncan's Multiple Range Test. Correlation analysis was carried out for the monthly capture rates of *B. dorsalis* and three monthly climatic factors: mean temperature, mean relative humidity, and total rainfall in study year.

3.12 Host plants relationship

All the possible regular and occasional host plants of the oriental fly around the selected study site were monitored. It was evident that some regular and occasional host plants were planted by the owner of land surrounding their living places. The principal agricultural host of *B. dorsalis* include banana, mango, guava, papaya, citrus, cucumber and tomato are among the major fruits crops cultivated in the experimental sites. Table 2 shows the approximate host points of the agricultural habitats where male oriental fruit flies were trapped.

Table 2. Approximate host points of the agricultural habitats where male

   oriental fruit flies were trapped

Month	Host points
Jan 12	90
Feb 12	90
March 12	150
April 12	180
May 12	180
Jun 12	180
July 12	120
Aug12	120
Sep 12	120
Oct 12	120
Nov 12	90
Dec 12	60

3.12 Statistical analysis

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance was done with the help of computer package MSTAT program (Gomez and Gomez 1976). The treatment means were separated by Duncan's Multiple Range Test (DMRT).

Chapter 4

Results and Discussion

RESULTS AND DISCUSSION

4.1 Seasonal Occurrence of Oriental fruit flies

The fortnightly captures of oriental fruit fly males were averaged on monthly basis in study area (Table 3). The prevalence of oriental fruit fly was found to be abundant in the study sites in Ganakbari area. The captured rate was increased from March and reached at higher peak in the month of May. The average number of adults captured per trap in 2012 varied from highest 314.8±12.15 (May) to lowest 30.5±8.6 (February) in Ganakbari, Savar, Dhaka. In this area *B. dorsalis* infestations occur round the year but peak population levels appear in the May to September. Numbers of the monthly capture were significantly related to the number of flies trapped in May to September when collections were peaked. The peak population levels mainly depended on the host fruiting availability. Result of this experiment was partially similar with the findigs of Makhmoor and Singh (1998) who reported that peak population (170.66 males/trap/week) of oriental fruit fly was observed in june in Indian occupied Kasmir area.

4.2 Relation with climatic factors

Monthly average air temperatures, rainfall and relative humidity are shown in the Table 3. A moderate positive correlations were found between monthly capture rates and the monthly average air temperatures (R^2=0.2726, Fig 1a) and total rainfall (R^2=0.113, Fig 1b). But no significant correlation

were found between monthly captured rates and relative humidity (R^2=0.065, Fig 1c) in all the areas.

Table 3. Number of (means ± SE) adult male oriental fruit fly, *Bactrocera dorsalis* captured per Mcphail trap in 2012 in Ganakbari area and monthly average temperature, relative humidity and total rainfall

month	No. of adult male captured(mean)	Ave Tem^{0}C	Ave RH %	Total rain fall (mm)
January	59±17.33fg	18.0 ±2.2	69.0 ± 6.3	23
February	30.5±8.6h	21.3 ± 3.3	68.0 ± 5.2	28
March	47.88±19.29g	27.6 ± 2.5	67.0 ± 6.3	79
April	59.5±15.09fg	30.2 ± 3.2	71.0 ± 6.8	105
May	314.8±12.15a	31.3 ± 3.6	70.0 ± 8.6	205
June	226.9±22.9b	30.7±3.2	80.0 ± 5.3	806
July	89.38±8.58e	27.3 ± 2.2	82.0 ± 8.2	1295
August	197.5±6.59c	28.2 ± 3.2	81.0 ± 3.5	1191

September	161.1±7.79d	29.5 ±2.8	82.0 ± 4.9	809
October	66.38±23.45f	26.2 ± 3.3	78.0 ± 5.6	170
November	205.5±8.79c	21.6 ± 3.5	64.0 ± 4.6	35
December	34.13±12.21h	20.3 ± 3.4	64.0 ± 6.7	12
LSD(0.05)	13.41			
CV(%)	10.83			

Means having the same letter in each column do not differ significantly at p<0.05 by DMRT.

$$y = 10.71x - 154.16$$
$$R^2 = 0.2726$$
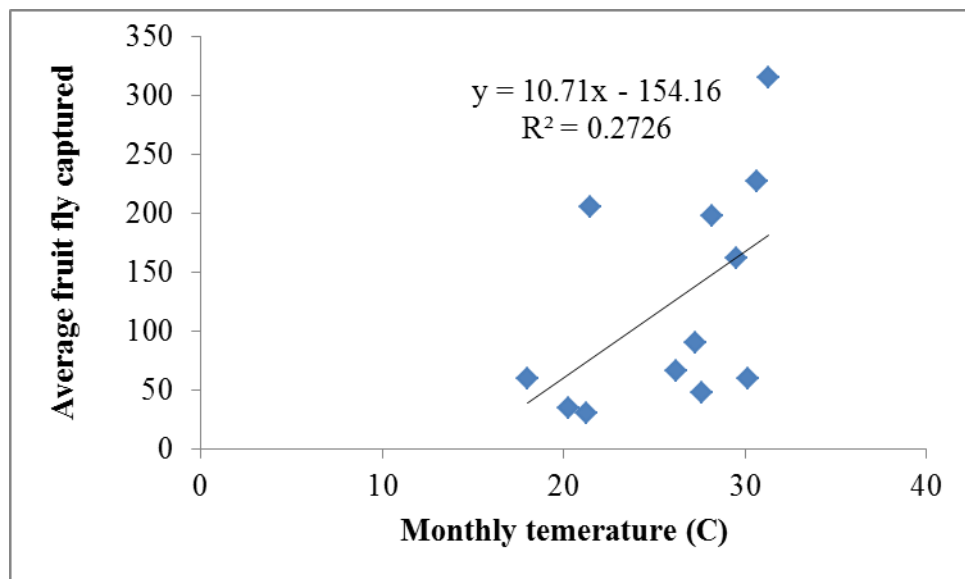
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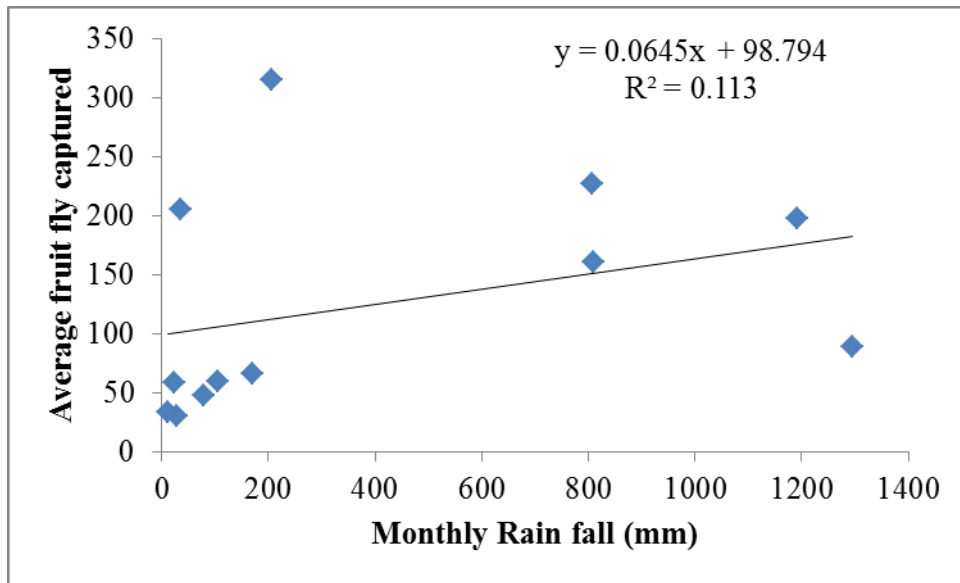
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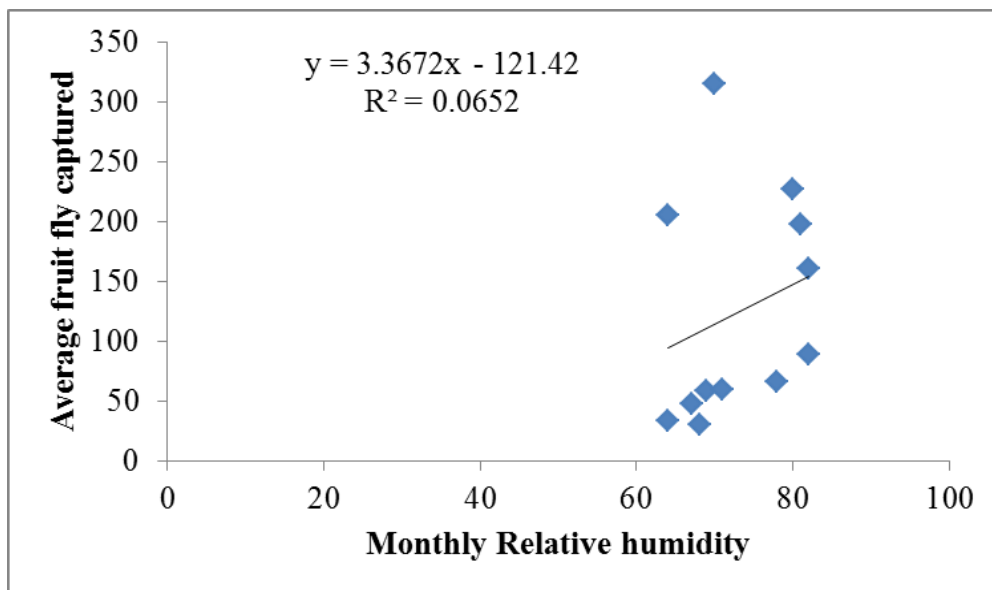
(c)

Figure 1. Regression line and correlation between air temperature (a),total rainfall (b) and relative humidity (c) with monthly average oriental fruit fly captured during 2012

4.3 Development of new larval diet

Larval diet of four different compositions were tried for rearing of oriental

fruit fly, *B.dorsalis*. Sweet potato paste was used as the main ingredients in

all four compositions of diet supplemented with wheat, rice and soya bran in different ratios. Artificial diet for rearing of *B.dorsalis* was prerared with locally available low cost ingredients. Expensive ingredients like yeast extract, casein hydrolysate, brewers yeast were not used in the current larval diet. At pH 4.87-4.89, the highest hatching percentage (83.91%) was observed and lowest hatching percentage (71.21%) was observed at 6.34-6.36 pH (Table 4). The highest larval duration was observed at 6.34-6.36 pH and the lowest at both 5.22-5.26 and 4.87-4.89 pH. The highest number of pupae was found at 5.22-5.26 pH and the lowest at 6.34-6.36 pH. Pupal duration was highest at 6.34-6.36 pH and lowest at 5.22-5.26 pH. In case of flier percentage and adult emergence percentage the best performance was observed at 5.22-5.26 pH (Table 4). The result indicates that the diet having 5.22-5.26 pH was found to be economical and suitable for mass scale rearing. Chan *et al*. (1998) reported that pH at >5.5 is essential to reduce the microbial activity in the diet. Because of the variation of biotic and abiotic factors some time the result do not supports the findings.

4.4 Pupal radiosensitivity

Radiation of different doses imposes effect on pupal duration ,% normal emergence, % deformed flies, % unemerged flies of *B. dorsalis*. The pupal duration at different ages and different doses varied significantly ($p < 0.05$). In all the cases, the higher doses of gamma radiation extended the pupal duration of the oriental fruit flies (Table 5 and 6). In every cases, normal

adult emergence percentage was highest in control batch and lowest was in the batch exposed to the highest dose of radiations. Adult emergence was affected considerably in pupal irradiated at different ages with varying doses of gamma radiation . Five days old pupae were relatively more radiosensitive than 6-days old. This result supports the findings of Balock (1963) who reported that radiosensitivity of pupae was found to be decreased as age increased (Balock 1963).

Table4

4.4.1 Effect of radiation on pupal duration

Pupal duration after imposing gamma radiation at different pupal ages are presented in (Table 5). For 5-days old pupae, the pupal duration of fruit flies were 6.67 ± 0.58, 7.667 ± 0.58, 8.67 ± 0.58, 10 ± 1 and 11.33 ± 1.52 for control, 30 Gy, 40 Gy, 50 Gy and 60 Gy, respectively. For 5-days old pupae, the highest pupal duration was found 11.33 ± 1.52 days irradiated with 60 Gy and the lowest was in control batch 6.67 ± 0.58 days. For 6-days old pupae, the pupal duration of fruit flies were as 7.33 ± 0.58, 8.33 ± 0.58, 9.33 ± 0.58 and 11 ± 1 for 30 Gy, 40 Gy, 50 Gy and 60 Gy, respectively but pupal duration of flies were found at control batch as 6.333 ± 0.58. For 6-days old pupae the highest pupal duration was found 11 ± 1 days irradiated with 60 Gy and the lowest was in control batch 6.33 ± 0.58 days. Between 5-days and 6-days old pupae the highest pupal duration was 11.33 ± 1.52 days irradiated with 60Gy. The result showed that the mean of pupal duration of flies is inversely correlated with the high dose of radiation.

Table 5. pupal duration after imposing different doses ofgamma radiation at 5 and 6 days old pupae of oriental fruit fly *Bactrocera dorsalis*

Dose(Gy)	5 Days	6days
control	6.667±0.58d	6.333±0.58d
30	7.667±0.58cd	7.333±0.58cd
40	8.667±0.58bc	8.333±0.58bc
50	10±1ab	9.333±0.58b
60	11.33±1.52a	11±1a
LSD(0.05)	1.649	1.287
CV	9.88	8.07

Means having the same letter in each column do not differ significantly at p<0.05 by DMRT.

4.4.2 Effect of radiation on normal adult emergence

Normal adult emergence after imposing gamma radiation at 5-days pupal ages were presented in table 6. For 5-days old pupae normal adult emergence of fruit flies were 87.67%, 58.08%, 16.34% and 0% for 30 Gy, 40 Gy, 50 Gy and 60 Gy, respectively. But at control batch normal emergence of fruit fly was 93.12%. Statistical analysis of percent normal emergence data showed that the highest percent normal emergence was obtained from control batch (93.12%) and the lowest from the pupae irradiated with 60Gy was 0% which was significantly different from other doses of radiation. The result shows that the percentage of normal adult emergence is inversely correlated with the high radiation.

4.4.3 Effect of radiation on Deformed flies emergence

Deformed flies emergence after imposing gamma radiation at 5-days pupal ages are presented in table 6. Abnormalities consisted mainly of wrinkled wings or wingless that failed to expand upon emergence. For 5-days old pupae deformed flies emergence of fruit flies were 4.59%, 18.38%, 15.35% and 5.55% for 30 Gy, 40 Gy, 50 Gy and 60 Gy, respectively. But in control batch deformed flies emergence of fruit fly was only 1.59%. Statistical analysis of percent deformed flies emergence data showed that the highest percent deformed fly emergence was found from the pupae irradiated with 40 Gy (18.38%) which was significantly different from other doses of radiation doses and the lowest deformed flies emergence found from the control batch 1.593%.

4.4.4 Effect of radiation on unemerged flies percentage

Percent unemerged flies after imposing gamma radiation on 5-days old pupae were also presented in table 6. For 5-days old pupae unemerged flies percentage of fruit flies were 7.733%, 23.55%, 68.31% and 94.44% for 30 Gy, 40 Gy, 50 Gy and 60 Gy, respectively. But in control batch unemerged flies percentage of fruit fly was 5.287%. Statistical analysis of percent unemerged flies data showed that the highest percent unemerged flies was found from the pupae irradiated with 60 Gy (94.44%) which was significantly different from other dose of radiation and the lowest percent deformed flies emergence was found 5.287% from the control batch.

Table 6. Normal emergence, deformed flies and unemergence percentage of oriental fruit fly *Bactrocera dorsalis* at different doses of radiation

Dose	%normal Emergence	%deformed flies	%unemerged flies
control	93.12a	1.593b	5.287d
30	87.67a	4.59b	7.733d
40	58.08b	18.38a	23.55c
50	16.34c	15.35a	68.31b
60	0d	5.553b	94.44a
LSD(0.05)	6.429	8.482	11.25
CV(%)	6.6	49.54	14.99

Means having the same letter in each column do not differ significantly at $p < 0.05$ by DMRT.

4.5 Optimization of sterility dose

The percentage of sterility and pupal recovery recorded at different doses of gamma irradiation are presented in Figure 2. For 5-days old pupae the pupal recoveries were 775±11.15, 448±6.33, 36±3.79, and 0 for 30, 40, 50 and 60 Gy dose levels, respectively. At control pupal recovery was 908±5.04. For 6-days old pupae the pupal recoveries were 885±8.66, 513±11.33, 48±37.69 and 3±0.58 for the 30, 40, 50 and 60 Gy dose levels, respectively. In control pupal recovery was 984±9.08. The sterility percentage increased gradually with the increase of radiation dose.

As because not a single oriental fruit fly emerged from the 5 days old pupae treated with 60 Gy and 60 Gy was selected as sterilizing dose.
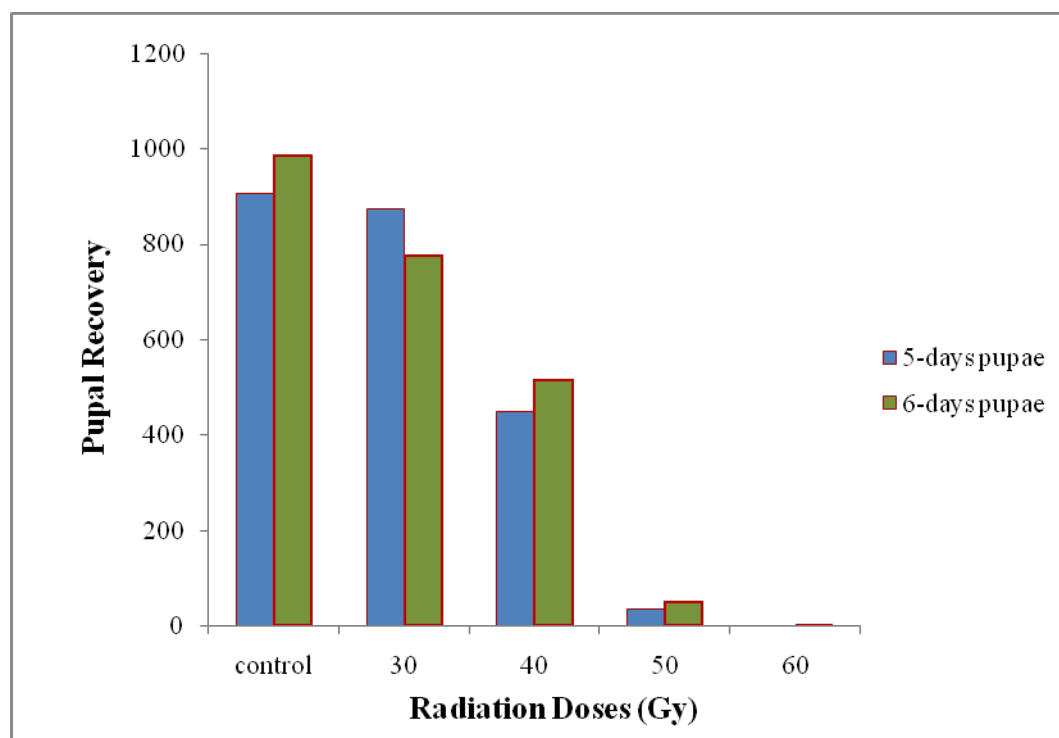
Figure 2. Mean pupal recovery of oriental fruit fly, *Bactrocera dorsalis* irradiated at different doses (Gy) of gamma radiation

4.6 Suppression experiments

4.6.1 Pupal recovery and percent normal emergence

In both laboratory and field cages trials number of pupae produced from different mating ratios of un-irradiated and irradiated males with virgin females of *Bactrocera dorsalis* are showed in Table 7 and 8. The result showed that the pupal recovery varied from 1:2, 1:5, 1:9 ratios and control batch. The highest pupal recovery was found in control batch and the lowest pupal recovery was found in 1:9 ratio. The pupal recovery at different ratios differs significantly in laboratory and field cage experiments. In case of

percent normal emergence the highest result was found in control and the lowest in 1:9 ratio (Table 9 and 10). The result showed that the number of pupal recovery and normal emergence percentage is inversely correlated with the ratio having high irradiated male oriental fruit fly i.e., the higher the ratio with irradiated males applied, the lower the number of pupal recovery percent normal emergence observed.

4.6.2 Percent deformed flies and unemergence percentage

Pecent deformed flies and unemergence percentage of oriental fruit fly from different mating ratios of un-irradiated and irradiated males with virgin females of *Bactrocera dorsalis* in both laboratory and field cage trials are shown in Table 11, 12,13 and 14. The result showed that the percent deformed and unemerged flies were varied from 1:2, 1:5, 1:9 ratios and control batch. The highest result was observed at 1:9 ratios and the lowest was found in control for both cases. The percent deformed and unemerged flies at different ratios differ significantly in laboratory and field cage experiment. The result showed that the percent deformed and unemerged flies were positively correlated with the ratio having high irradiated male fruit fly i.e. the higher the ratio with irradiated males applied, the higher the percent of deformed and unemerged flies observed. The results have some similarities with the observation of Toledo (2010). The classic over flooding ratio of 9 sterile male to 1 fertile male as proposed by Singh (2000).

Chapter 5

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at the in the laboratory and experimental field of Insect Biotechnology Division (IBD), Institute of Food and Rediation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka during January 2012 to February 2013 to establish the Sterile Insect Technique (SIT) method for controlling *Bactrocera dorsalis* (Hendel)).

In the resent study an attempt was made to utilize different ratios between irradiated and unirradiated male of oriental fruit flies such as 1:1, 1:2, 1:5 and 1:9 (unirradiated male: irradiated male) were allowed to mate with a fixed number of unirradiated vergine females of oriental fruit flies in different cages to find out the rate of suppression against wild population. Parameters like pupal duration, number of pupae production,% normal emergence,% deformed flies,% unemerged flies were recorded. In case of artificial larval diet hatching percentage, larval and pupal duration, number of pupae production, flier percentage and adult percent emergence were recorded. The mean difference among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

In case of artificial larval diet although the highest hatching percentage was observed from the 4.87-4.89 pH but based on highest no. of pupae production, the lowest larval and pupal duration, flier percentage and adult percent emergence at 5.22-5.26 pH was found to be economical and suitable for mass scale rearing.

The highest pupal duration was 11.33 ± 1.52 found from 5 days old pupae treated with 60 Gy and in case of control it was the lowest (6.667 ± 0.58). The highest pupal recovery was at control (885 ± 8.66) from 6 days old pupae. At 60 Gy from 5 days old pupae there was no pupal recovery. Percent deformed flies were the highest at 40 Gy dose (18.38%) where in contro it was 1.593%. In case of percent unemerged flies the highest result was found from 60 Gy (94.44%) and the lowest from control batch (5.28%). The ratio with large group of treated male caused decreasing characters except in case of pupal duration (days), deformed flies (%) and unmerged flies (%). In these cases the result showed the increasing rate with increasing number of treated male. The pupation rate and normal adult emergence of oriental fruit flies from 1:2 to 1:9 ratios showed a gradual increase in accordance with the increasing number of irradiated male. Every time the highest observation was found in control and the lowest was at 1:9 ratio.

The pupal recovery varied from 280.33±18.01 to 8.667±3.06 in laboratory cage trials and 937±18.25 to 44±7.21 in field cage trials due to control to 1:9 ratio. Normal percent adult emergence was 84.16% and 97.96% at 1:2 ratio while it reached to 47.28 % and 52.61% at 1:9 for laboratory and field cage trials respectively. In control it was 94.28% and 99.96%. Percent deformed and unemerged flies were positively correlated with the gradual increasing ratio i.e. the higher the ratio with irradiated males released, the higher the percentages of deformed flies and unemergence.

In case of percent deformed flies the best result was found from 1:9 ratio (35.51% and 29,86%) for laboratory and field cage trials, respectively. Percent unemerged fruit fly also highest in 1:9 ratio (25.2% and 18.61%) for laboratory and field cage trials, respectively. In control it was 0% in the field cage trials. Data generated from this study in respect of sterility dose, pupal duration and on the ratios of irradiated males of *Bactrocera dorsalis* could be used as a preliminary basis for the field application of sterile insect technique to suppress the wild population of this fruit fly.

RECOMMENDATIONS

❖ In case of artificial larval diet 5.22-5.26 pH was found to be economical and suitable for mass scale rearing and this pH level may be recommended for development of artificial larval diet.

❖ It can be stated that with a view to control and suppress the population of wild oriental fruit flies one may release irradiated (treated with 60 Gy) males of *Bactrocera dorsalis* in nature at 1:9 ratio (unirradiated male: irradiated male).
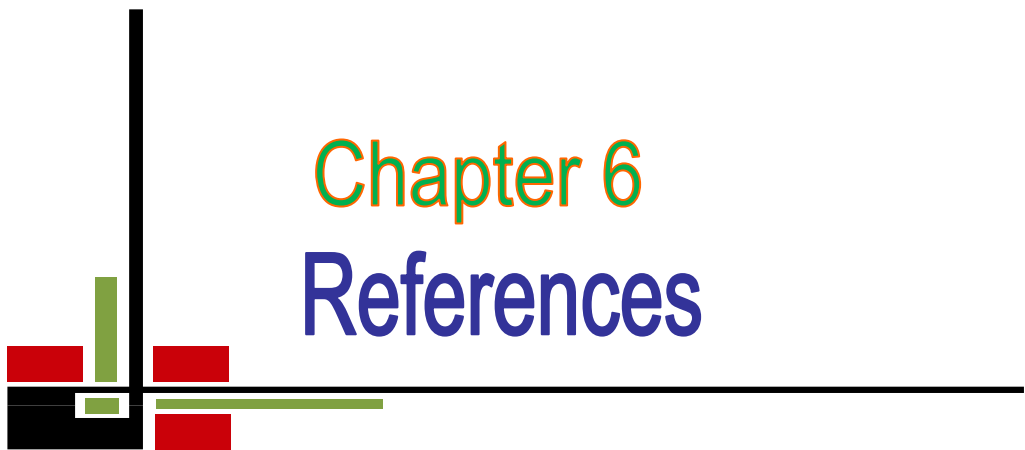
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Appendices

APPENDICES

Appendix I. Map showing the major fruit fly oriented area of Bangladesh

Appendix II. Mean square values for Number of adult male oriental fruit fly,
***Bactrocera dorsalis* captured per Mcphail trap in 2012**

Sources of variation	Degrees of freedom	No. of adult oriental fruit fly captured per trap
Replication	7	605.83
Factor A	11	69752.34*
Error	77	181.40

*significant at 5% level of probability,
NS- Non significant

Appendix III. Mean square values for effect of pH on the cost effective artificial diet for oriental fruit fly *Bactrocera dorsalis* (Herdel) Larvae

Sources of variation	Degrees of freedom	Hatching percentage	Larval duration	No. of pupae	pupal duration	flier %	Adult emergence %
Replication	2	6.63	1.58	52204.75	1.083	92.33	1.22
ratios	3	91.65*	8.52*	72006.778*	3.33*	62.22^{NS}	28.7*
Error	6	0.299	.36	10186.19	0.083	17.88	1.42

*significant at 5% level of probability,
NS- Non significant

Appendix IV. Mean square values for pupal duration after imposing gamma radiation at different pupal ages of oriental fruit fly *Bactrocera dorsalis*

Sources of variation	Degrees of freedom	Pupal duration after imposing ganna radiation .	
		5 days	6 days
Replication	2	1.267	0.467
Factor A	4	10.267*	9.767*
Error	8	0.767	0.467

*significant at 5% level of probability,
NS- Non significant

Appendix V. Mean square values for Normal emergence, deformed flies and unemergence percentage of oriental fruit fly *Bactrocera dorsalis* **at different radiation doses**

Sources of variation	Degrees of freedom	%normal Emergence	%deformed flies	%unemerged flies
Replication	2	2.711	21.86	9.28
Factor A	4	5228.24*	160.82*	4711.42*
Error	8	11.34	20.29	35.71

*significant at 5% level of probability,
NS- Non significant

Appendix VI. Mean square values for Number of purae pupae produced from different mating ratios of un- irradiated and irradiated males with virgin females (60) of *Bactrocera dorsalis* in laboratory trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	15th	16th	17th	18th	19th	20th
Replication	2	4785.08	268	141.75	1862.58	435.58	375.25	497.58
ratios	3	15300.44^{NS}	18542.88*	38299.41*	16590.77*	15485.41*	19335.41*	18750.44*
Error	6	4802.19	135.22	538.41	393.02	854.91	103.91	422.02

*significant at 5% level of probability,
NS- Non significant

Appendix VII. Mean square values for. number of pupae produced from differen mating ratios of unirradiated and   irradiated males with virgin females (300) of oriental fruit fly *Bactrocera dorsalis* in field cage trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	15th	16th	17th	18th	19th	20th
Replication	2	138.25	266.58	23.08	285.75	163.58	90.58	91.58
ratios	3	422816.22*	417942.33*	376253.86*	421319.86*	470162.52*	380809.86*	477360.55*
Error	6	100.139	144.583	430.19	702.86	729.02	202.36	467.13

*significant at 5% level of probability,
NS- Non significant

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Appendix VIII. Mean square values for Normal emergence percentage of oriental fruit fly *Bactrocera dorsalis* from different mating rations in laboratory trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	**15th**	**16th**	**17th**	**18th**	**19th**	**20th**
Replication	2	745.217	0.45	16.22	8.706	5.99	11.26	0.533
ratios	3	613.31^{NS}	1230.27*	1169.42*	1155.33*	1212.96*	973.09*	1001.48*
Error	6	630.263	3.473	6.08	7.34	10.64	8.847	4.24

*significant at 5% level of probability,
NS- Non significant

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Appendix IX. Mean square values for Normal emergence percentage of oriental fruit fly *Bactrocera dorsalis* from different mating ratios in field cage trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	15th	16th	17th	18th	19th	20th
Replication	2	0.303	21.26	221.36	10.96	11.96	0.5	0.48
ratios	3	1589.23*	1354.85*	2039.97*	1132.95*	1276.68*	1364.07*	1525.13*
Error	6	0.26	10.61	222.47	10.97	2.88	8.73	2.93

*significant at 5% level of probability,
NS- Non significant

Appendix X. Mean square values for Deformed flies percentage of oriental fruit fly *Bactrocera dorsalis* from different mating ratios in laboratory trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	15th	16th	17th	18th	19th	20th
Replication	2	0.44	3.95	3.79	1.66	6.66	7.12	26.52
ratios	3	524.94*	648.94*	553.72*	507.09*	471.27*	528.47*	373.11*
Error	6	8.13	2.966	3.19	2.42	3.87	12.07	19.27

*significant at 5% level of probability,
NS- Non significant

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Appendix XI. Mean square values for. Deformed flies percentage of oriental fruit fly *Bactrocera dorsalis* from different mating ratios in field cage trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	15th	16th	17th	18th	19th	20th
Replication	2	2.64	7.21	3.56	2.77	1.47	2.27	0.31
ratios	3	616.38*	528.57*	602.33*	552.98*	669.06*	497.61*	501.46*
Error	6	1.22	4.43	1.59	7.38	0.73	6	1.36

*significant at 5% level of probability,
NS- Non significant

Appendix XII. Mean square values for Unemergence percentage of oriental fruit fly *Bactrocera dorsalis* from different mating ratios in laboratory trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	15th	16th	17th	18th	19th	20th
Replication	2	5.17	2.83	9.74	4.95	2.28	4.09	21.4
ratios	3	263.09*	84.13*	113.05*	133.41*	164.58*	89.04*	236.36*
Error	6	10.53	5.33	2.95	5.98	14.69	7.6	

*significant at 5% level of probability,
NS- Non significant

Appendix XIII. Mean square values for Unemergence percentage of oriental fruit fly *Bactrocera dorsalis* from different mating ratios in field cage trials

Sources of variation	Degrees of freedom	Adult age(days)						
		14th	**15th**	**16th**	**17th**	**18th**	**19th**	**20th**
Replication	2	0.43	8.1	3.65	13.41	6.15	1.36	0.63
ratios	3	231.83*	194.63*	169.3*	128.5*1	100.05*	216.05*	261.29*
Error	6	1.56	4.14	3.36	7.31	3.1	0.89	2.37

*significant at 5% level of probability,
NS- Non significant