DIVERSITY AND HOST PREFERENCE OF TEPHRITID FRUIT FLY SPECIES INFESTING CUCURBIT AND MAJOR HORTICULTURAL CROPS GROWN IN THE LOWER COASTAL KENYA

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DECLARATION

This is my original work and has not been submitted for award of a degree in another university

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Appendix I Declaration Form for Students

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DEDICATION

I dedicate this thesis to my parents Paul Kambura and Faith Ng'era for their support and dedication to ensure that I acquired the deserved education. I also dedicate this thesis to my sister, relatives and friends for their social and moral support and their continued prayers throughout the study.

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ABSTRACT

Tephritid fruit flies are among the notorious pests of horticultural crops in Kenya and have been documented to cause yield losses of 30-100%. Tephritid fruit flies from the *Bactrocera* and *Dacus* genera have been identified on cucurbit farm traps. However, their losses have not been quantified. Therefore, this study was conducted to determine the occurrence, diversity and host preference of tephritid fruit flies infesting cucurbit and other horticultural crops in coastal Kenya. Infested cucurbit and major horticultural crops were randomly sampled and incubated in the laboratory. The emerging adults from the recovered pupae were identified based on their morphology. Host preference tests of the dominant and most prevalent fruit fly species that is *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* were conducted on courgettes (*Cucurbita pepo*), butternut (*Cucurbita moschata*), cucumber (*Cucumis sativus*) and watermelon (*Citrillus lanatus*). Data on the number of pupae recovered, pupae/ml, percent adult emergence, percent deformity, percent sex ratio and tibia measurements of the insects from each host plant was collected. Mass rearing of the dominant fruit fly species was carried out on the suitable and readily available host plant. Pupae recovered, weight of pupae, percent adult emergence, fecundity, percent egg hatch and percent flier were observed to determine the suitability of using butternut in the mass rearing of the three species to enable laboratory studies of these species.

Bactrocera cucurbitae, *Dacus bivitattus*, *Dacus ciliatus* and *Dacus vertebratus* were the major tephritid fruit flies found infesting cucurbit in coastal Kenya. In addition, *B. cucurbitae* and *D. ciliatus* were also found to attack *Solanaceae*, *Rutaceae*, *Myrtaceae*, and *Anarcadiaceae* crops. The most dominant fruit flies identified in cucurbit were *Bactocera cucurbitae*, *D. bivitattus* and *D. ciliatus*. Watermelon, butternut, courgettes and cucumbers were the most preferred crops by

B. cucurbitae and *D. bivitattus* while *D. ciliatus* mostly preferred watermelon, courgettes, butternut and cucumber. Butternut was found to be the most suitable host plant for rearing *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* in the absence of an artificial diet. These findings show that *B. cucurbitae*, *D. bivitattus, D. ciliatus* and *D. vertebratus* are significant pests of cucurbit. In addi, *B. cucurbitae* and *D. ciliatus* are also major pests of *Solanaceae, Rutaceae, Myrtaceae*, and *Anarcadiaceae*. Hence, pest management programs should focus in the management of these tephritid fruit flies in horticultural farms. The host preference study indicated that watermelon was the preferred host plant for *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus*. However, butternut was the suitable host for the mass rearing of *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus*. Therefore, bioecological studies such as developing artificial diet for the mass rearing of these species should consider using butternut as an ingredient in the diet.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Agriculture is among the largest industry in the world according to the World Trade Organization (2014). It is the primary source of food for human beings and fodder for animals (WTO, 2014). It plays a significant role in the world's economy by employing one billion people while generating 1.3 trillion dollars' worth of food annually (WTO, 2010). In Africa, agriculture is a significant economic activity. It contributes to more than 30% development in several countries especially in the Sub- Saharan Africa and employs 65% of Africa's labor force (FAO, 2012). In Kenya, agriculture; crop production and livestock keeping is the mainstay of the economy (Kenya Economic Report, 2013). It directly employs 75% of the national labor force and contributes approximately 25% of the gross domestic product (Alila and Atieno, 2006). It also provides 45% of the government's revenue, 75% of the raw materials and 60% of the exports from Kenya (Murithi, 2009).

Horticulture is among the primary important subsectors in the Kenyan agriculture. It is a core contributor in achieving food security, acquiring raw materials and in foreign exchange earning that are essential indicators of a stable economy (National Horticultural Policy, 2010). Despite the enormous importance of the horticultural sector, it is faced by countless biotic and abiotic constraints (Ndung'u, 2010). Among the biotic factors, pests and diseases have consistently ranked high as significant limitations to increasing productivity by causing 25-35% yield losses (Nyakundi *et al.*, 2012).

Cucurbit are among the major horticultural crops grown in Kenya and exported abroad for their nutritional value and economic significance as foreign exchange earners (Njoroge, 2012). The various major species grown in Kenya are butternut (*Cucurbita moschata*), pumpkin (*Cucurbita* *maxima*), cucumber (*Cucumis sativus*), courgettes (*Cucurbita pepo*), and watermelon (*Citrullus lanatus*) (HCDA, 2011).

Pests are classified as of primary economic importance in cucurbit production because of the high cost of pest management in controlling them and the reduction which they cause in crop production (Davis *et al.*, 2008; Sapkota, 2010).Various arthropod pests are reported to attack cucurbit, and fruit flies of the family Tephritidae have been noted as the most notorious (Geurts *et al.,* 2012; Stibick 2004; Ryckewaert *et al.,* 2010). This is because of the direct and indirect losses which they cause (Ekesi and Mohamed, 2011; Price,1999).

Worldwide, tephrtitids are also reported to cause significant losses to other horticultural crop families of economic importance apart from the cucurbit family. These include *Solanaceae* (Mziray *et al.*, 2010; Mcquate, 2008; Ramadan and Messing, 2003;), *Rutaceae* (Goergen *et al.*, 2011; Thomas and Shellie, 2000; White and Wang, 2009), *Myrtaceae* (Souza-Filho *et al.*, 2009; Marsaro Júnior *et al.*, 2013), and *Anarcadiaceae* (Mwatawala *et al.*, 2009; Abdullahi *et al.*, 2011). In Kenya, tephritid's have also been identified as pests of these families (Rwomushana *et al.*, 2008).

The genus *Bactrocera*, *Ceratitis* and *Dacus* have been documented to be the primary tephritid fruit flies that cause the highest percentage of losses in Africa (Jose *et al.*, 2013; Badii *et al.*, 2015). According to Ekesi and Billah (2007), *B. cucurbitae* and *Dacus* sp. are the major tephritid pests of cucurbit. Although the tephritid flies are associated with losses in cucurbit farms, there is no record on the quantification of their losses, host preference and mass rearing procedure in Kenya. As a result of the quarantine nature of these fruit fly species, it is important to carry out bioecological studies on *B. cucurbitae* and *Dacus* sp. to reduce their losses in horticultural crops in Kenya though providing information that would improve the management of this losses.

1. 2 Problem statement

Cucurbit are generally regarded as major horticultural crops in Kenya because of the role which they play in improving food and nutritional security (Njoroge, 2012). According to the Horticultural Crop Development Authority report (2012), cucurbit are also key foreign exchange earners in the horticulture industry. However, the family faces significant constraints because of tephritid fruit flies from the *Dacus* species and *B. cucurbitae*.

Certain regions of the world such as India have reported crop losses of more than 30% in cucurbit farms caused by tephritid fruit flies from the genus *Dacus* (Dhillon *et al.,* 2005; Stonehouse *et al.,* 2007). In Kenya, tephritid fruit flies are ranked as quarantine pests and have led to the banning of some crops such as the avocado from being exported to other countries like South Africa (HCDA, 2010). Due to the quarantine nature of this family, it is important to understand each species damage, yield and market losses to enable their management.

Although *Dacus* species and *B. cucurbitae* have been identified on horticultural farm traps as significant pests (Ekesi and Billah, 2007) in the country, very little is known about their interactions with host crops and the resultant consequences to host crop yield production and performance. There is an urgent need to determine the diversity of tephritid fruit fly species that attack cucurbit and the principal horticultural family crops which are grown locally in Kenya and the infestation indices to these crops. It is also important to determine host preferences in order to assist in their management and also develop a good mass rearing technique which will enable further studies of these pests in the laboratory.

1. 3 Justification

Cucurbitaceae, Solanaceae, Rutaceae, Myrtaceae, and *Anarcadiaceae* are of great importance in Kenya as sources of food and revenue (Bisognin, 2002; Sindiga *et al.,* 1995). However, their production faces a significant challenge of fruit flies (Tephritidae) from the *Dacus* species that result to reduced crop yield (Ekesi and Billah, 2007) and trade restrictions (Mugure, 2012). Though the *Dacus* genus is reported to be a major pest of horticultural production, no information exists on the amount of damage caused by different *Dacus* species in Kenya.

Globally, only a few species of the Tephritidae fruit fly family have been reared successfully in the laboratory using either artificial diet or host plants. Among those reared successfully in the laboratory include *Bactocera invadens* (Ekesi *et al.,* 2007), *Bactrocera dorsalis* and *Ceratitis capitata*, (Chang et al., 2007), *Bactrocera cucurbitae* (Chang *et al.,* 2004) and the American fruit fly, *Anastrepha fraterculus* (Sobrinho *et al.,* 2006). This has enabled further research of the species in the laboratory that has assisted in the development of good pest management strategies. *Dacus* species consist of most species which are not reared in the laboratory. An information gap exists on how to successfully rear *Dacus* species in the laboratory using either artificial diet or host plants. Therefore, developing an efficient mass rearing procedure in the laboratory for *Dacus* species will assist in future research on the *Dacus* genus. Understanding the host preference of damaging *Dacus* species will contribute to the development of appropriate pest management programs that will assist farmers in reducing losses on their horticultural farms as a result of *Dacus* species.

1. 4 Objectives

The broad objective of the study was to contribute to the improved management of Tephrid fruit flies in cucurbits through understanding their diversity and host preference for increased cucurbit production.

The specific objectives were:

i. To determine the diversity of tephritid fruit fly species infesting cucurbit and other major horticultural crops in coastal Kenya

ii. To determine the host preference and mass rearing procedure of the dominant tephritid fruit fly species.

1. 5 Research hypothesis

1. There is diversity of tephritid fruit fly species infesting horticultural crops in Coastal Kenya due to the region being a major point of entry into the country from other parts of the world.
2. Tephritid fruit fly species infesting cucurbit in Kenya have host preferences due to the polyphagous nature of this family.

CHAPTER TWO: LITERATURE REVIEW

2.1 Economic importance of horticulture in Sub-Saharan Africa (SSA)

In Sub-Saharan Africa, 70% of the population lives in the rural areas and they practice agriculture as their principal activity due to its importance as a source of food and income (Thornton *et al.,* 2011). The Agricultural sector contributes to about 30% of the GDP while employing 65% of the labor force (Diao *et al.,* 2010). In Sub-Saharan Africa, the horticultural sub-sector has a significant role as a leading supplier of horticultural produce in the United Kingdom (UK) supermarkets (Legge, 2006). Kenya is one of the major exporters of the horticultural produce to the European markets with exporting shares of 32% in UK, 32% in Netherlands, 17% in France, 11% in Germany and 8% in other European countries (HCDA, 2009).

In Kenya, the horticultural industry is the second foreign exchange earner after tourism. In 2011 it contributed 91.2 billion KES on exports (HCDA, 2012). However, Kenya's horticultural industry faces significant economic challenges; poor market and production systems, rising cost of inputs such as fertilizers and agrochemicals, lack of technical knowhow, germplasm conservation and the changing environmental factors (biotic and abiotic) (Muthoka and Ogutu, 2014; Ekesi, 2010).

2. 2 Cucurbit and their production in Kenya

Cucurbit belongs to the family *Cucurbitaceae* which is a taxonomic unit containing essential fruits and vegetables that act as sources of vitamins, minerals and fiber (Bisognin, 2002). The family has no close relatives, and the cultivated ones are found in the subfamily *cucurbitoideae* (Malley, 2008). Their principal characteristics are that they are a trailing or vining, tendril bearing, frost sensitive, annuals and monoecious (Wehner and Maynard, 2003). The crops thrive well in warm-seasons, with optimum production occurring at the temperatures of 23-32°C during the day and 15-21°C at night (Davis *et al.*, 2008). Loam, sandy loam, and loam sandy are the most favorable soil types for the growth of cucurbit (Wehner and Maynard, 2003). Cucurbit are among the key horticultural crops grown in Kenya and are mainly grown in Kilifi, Taita/Taveta, Kisii, Meru, Kiambu and Migori (HCDA, 2012) (Figure 2.1).

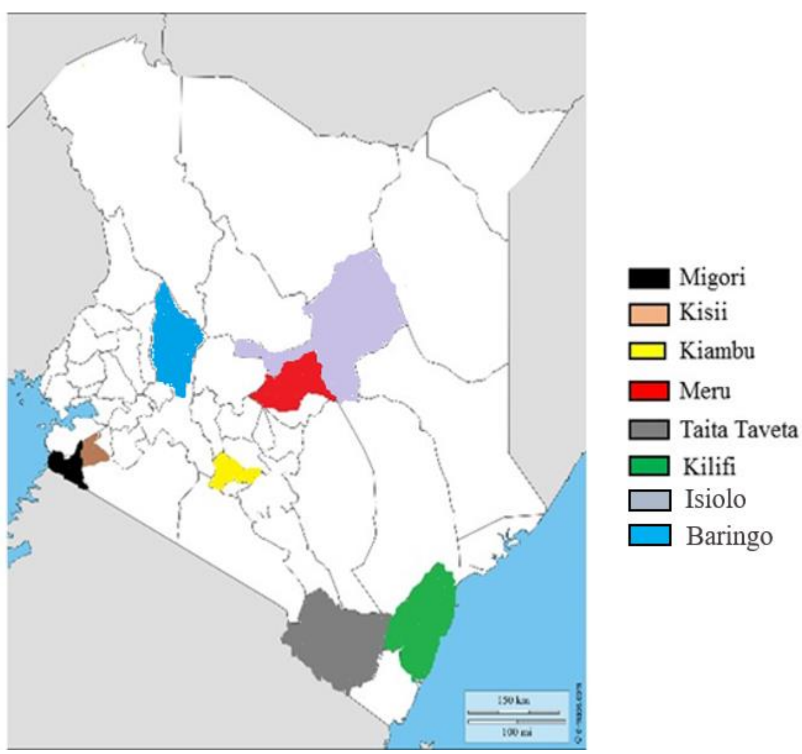

Figure 2.1. Cucurbit growing regions in Kenya (HCDA: 2009)

However, among these regions, Coastal Kenya stands out in its importance as an entry point into the country and can act as a pathway for the introduction of new pests and diseases. *Bactrocera invadens*, a tephritid fruit fly, was first identified in Coastal Kenya (Lux *et al.,* 2003). It has caused a major economic damage in the horticultural industry because of the phytosanitary concerns and hence the need for periodic surveillance studies to grasp the status of tephritid fruit flies in Kenya. Based on the Kenya meteorological department, Coastal Kenya has a tropical climate with high rainfall and temperatures throughout the year. Cucurbit have been documented to perform well in such climatic conditions (Bisognin, 2002). The temperatures in the Coast range from 28°C±5 which have also been described to be the optimal temperatures for the survival and development of tephritid fruit fly species (Rwomushana *et al.,* 2008; Ekesi and Mohamed, 2011; Ekesi *et al.,* 2007).

Cucurbit production in Kenya is increasing annually probably due to the growing demand in line with the family's economic and nutritional value as observed in the table below (Table 2.1). However, it is difficult to get individual statistical data of the changes in each individual cucurbit crop production as they are always grouped together in reports. Comparisons in Kenya on four major cucurbit production figures between years 2005 and 2009 demonstrate that there was an increase in their production (Table 2.1). This probably reflects growth in the demand of these crops (HCDA, 2009). Cucurbit farming in Kenya like any other around the world is challenged by both biotic and abiotic stresses. However, tephritid fruit flies are recorded as the most notorious pests when it comes to cucurbit losses (Billah *et al.,* 2009; Badii *et al.,* 2015).

Table 2.1. Cucurbit production in Kenya in 2005 and 2009

Cucurbit species	2005		2009	
	Quantity (Tons)	Size of land (ha)	Quantity (Tons)	Size of land (ha)
Cucumber	25,900	27	43,775	27.5
Courgettes	55,750	223	209,754	553
Butternut	56,000	56	176,696	532
Watermelon	879,600	1,466	946,191	1,493

Source: HCDA, 2009

2. 3 Pests of cucurbits

Many pests and diseases attack cucurbit crops. Viral diseases such as cucumber mosaic virus and other potyviruses (Jacquemond, 2012; Abou-Jawdah *et al.*, 2000; Wang *et al.*, 2002); bacteria diseases such as vine decline diseases and bacterial wilt (Zeriouh *et al.*, 2011; Bruton *et al.*, 2007) and fungal diseases such as powdery mildew (Jahn *et al.*, 2002; King *et al.*, 2008) contribute to yield losses in cucurbit farms. Insect pests such as aphids (Emden *et al.*, 2007), thrips (Messelink *et al.*, 2008), beetles (Yardim *et al.*, 2006), whiteflies (Messelink *et al.*, 2008) and tephritid flies (de Meyer *et al.*, 2012) are reported as the major pests of cucurbit. Tephritid fruit flies are ranked high as the most notorious pests of economic importance on cucurbit and other horticultural crop families grown in Kenya (Dhillon *et al.*, 2005; Sapkota, 2010; FAO, 2012; Ekesi and Billah , 2007).

2. 4 Classification and distribution of Tephritid fruit flies

Tephritid fruit flies belong to the order Diptera, the Superfamily Tephritoidae, the Family Tephritidae and the Subfamily Dacinae. The family Tephritidae is the largest family of the

Diptera order (Clarke *et al.,* 2002; Drew, 1989) and has approximately 4000 species of tephritid fruit flies (Dhillon *et al.,* 2005). This family is categorized as one of those having the most damaging pests in the horticultural industry (Ekesi, 2010; Meyer, 2013; Geurts *et al.,* 2012; Stibick 2004; Stonehouse *et al.,* 2007; FAO, 2012). The genera of *Bactocera*, *Dacus*, *Ceratitis* and *Trirhithrum*, *Anastrepha* and *Rhagoletis* contain the most important species of economic importance on horticultural crops worldwide (White and Elson-Harris, 1992). Tephritid fruit flies are distributed in the tropical, subtropical and temperate regions of the world (Ansari *et al.*, 2012; Figure 2.2).

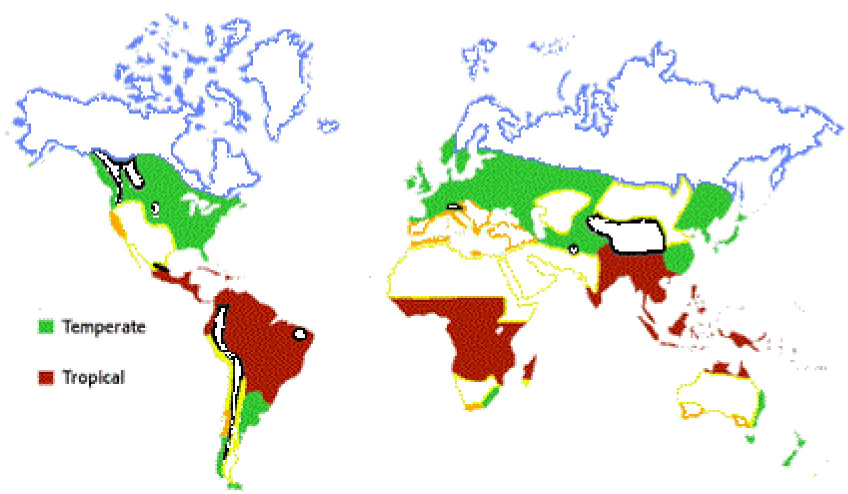

Figure 2.2. Distribution of Tephritid Fruit Flies in the World (Source Ansari et al., 2012)

According to Ekesi and Billah (2007) Sub-Saharan Africa has the highest potential of Tephritid fruit flies. Species from the *Bactrocera*, *Dacus*, *Ceratitis* and *Trihithrum* genus are classified as important insect pests in Africa (Billah *et al*., 2009; Virgilio *et al.*, 2013; Virgilio *et al.*, 2009). Among the *Bactrocera* species identified, *B. latifrons*, *B. cucurbitae*, *B. invadens* and *B. zonata* are the common species in Sub- Saharan Africa (Badii *et al.,* 2015) (Figure 2.3). However, *B.*

zonata is recorded in Egypt and Indian Ocean islands (Billah *et al.,* 2009), while *B. latifrons* is only recorded in Kenya and Tanzania (Mziray *et al.*, 2010; Ekesi and Billah, 2007). *Dacus* species recorded in Africa are *D. bivitattus*, *D. ciliatus*, *D. frontalis*, *D. lounsburyii*, *D. punctatifrons* and *D. vertebratus* (Figure 2.4). These species have all been identified in Sub-Saharan Africa as pests of cucurbit (White and Elson-Harris, 1992). From the genus *Ceratitis*, C *cosyra*, *C. rosa* Karsch, *C. fasciventris* Bezzi, *C. anonae* Graham, *C. capitata*, *C. quinaria* and *C. rubivora* (Coquillet) have been recorded as of economic importance in Sub-Saharan Africa(Badii *et al.*, 2015; Billah *et al.,* 2009) (Figure 2.5).

The morphological characteristics of the five tephritid genera varies according to species and individuals, with the wings of most species being colored with yellow, black or brown strips or spots or a combination of all (Christenson and Foote, 1960; White and Wang, 2009). *Bactrocera* is the most common genera of the family Tephritidae consisting of 500 described species - divided into 28 subgenera (Clarke *et al.,* 2002). However, in Kenya the most common are *B. invadens*, *B. cucurbitae* and *B. latifrons* (Billah *et al.,* 2009). The genera has the following common characteristics; oval abdomen, the lateral and medial postsutural are yellow, and the scutum is black (Prabhakar *et al.,* 2012).

Dacus species from the tephritidae family are also significant economic pests of horticultural crops (Ntonifor and Okolle, 2006). However, *D. bivittatus* Bigot, *D. ciliatus* Loew, *D. frontalis* Becker, *D. lounsburyii* coquillett, *D. punctatifrons* Karsch, and *D. vertebrates* Bezzi are the most prevalent in Kenya. They have common characteristics of petiolate abdomen; scutum has no yellow or orange stripes (Ntonifor and Okolle 2006; Billah *et al.,* 2009; Prabhakar *et al.,* 2012).

Ceratitis genus consist of 89 species divided into six subgenera (Barr and McPheron, 2006). However, *C. cosyra* Walker, *C. rosa* Karsch, *C. fasciventris* Bezzi, *C. anonae* Graham, *C. capitata* Wiedemann and *C. rubivora* coquillett are the most common in Kenya (Virgilio *et al.*, 2013; Wharton *et al.*, 2000; BaiNi *et al.*, 2009; Copeland *et al.*, 2006). The genera have the following characteristics; the scutellum has yellow areas (wavy yellow bands), presence of an isolated preapical cross band, basal cells of wings with spots and fleck-shaped marks *(Billah et al.*, 2009). *Trihithrum* species is a tephritidae fruit fly although little information exists about it. Common species recorded are *T. coffeae* Bezzi and *T.nigerrimum* and are characterized by small, scutellum almost entirely brown black at most with yellow spots to the scutellar setae. The basal cells are not reticulate like those of *Ceratitis* and *Trihithrum* species have sexual dimorphism (Billah *et al.*, 2009).

Bactrocera invadens *Bactrocera cucurbitae* *Bactrocera latifrons*

Figure 2.3. Tephritid fruit flies from the genus *Bactrocera* of economic importance in Kenya. (Photos by R. Copeland *icipe*)

Dacus bivittatus

Dacus ciliatus

Dacus vertebratus

Dacus punctatifrons

Dacus frontalis

Dacus lounsburyii

Figure 2.4. Tephritid fruit flies from the genus *Dacus* of economic importance in Kenya. (Photos by R. Copeland *icipe*)

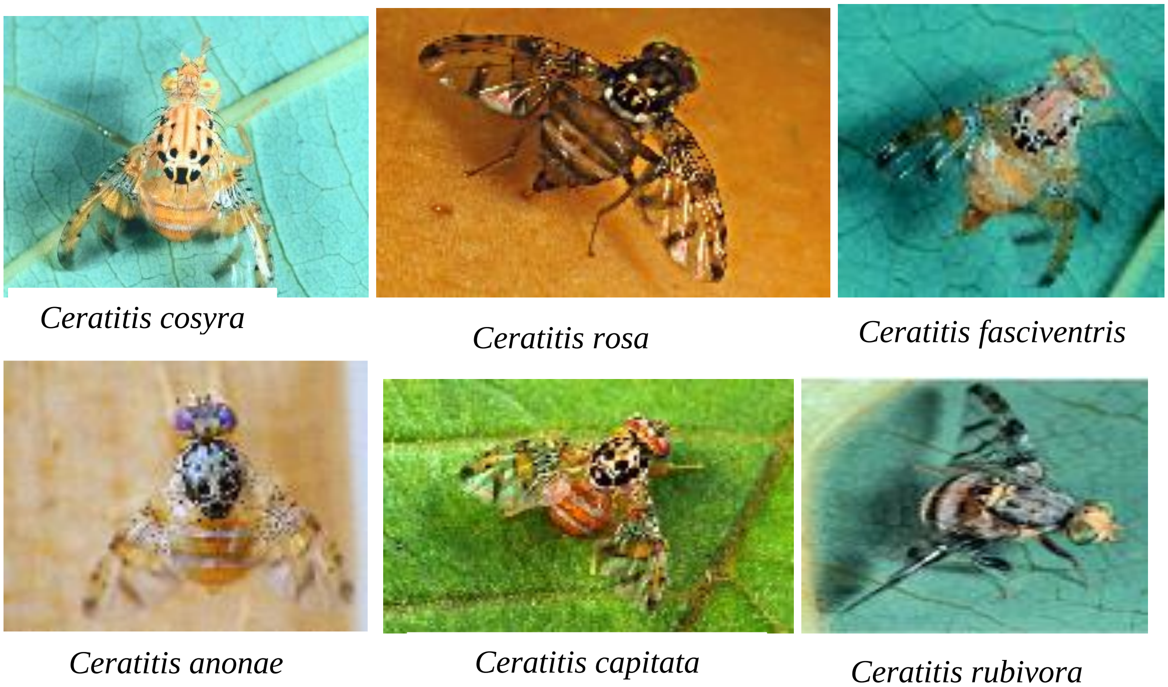

Figure 2.5. Tephritid fruit flies of the genus *Ceratitis* of economic importance in Kenya. (Photos by R. Copeland *icipe*)

2. 5 The biology of Tephritid fruit flies

Fruit fly (Tephritidae) adult and the larvae have different feeding and living habits (Frias, 2008). However, the adult determines the feeding site for the larvae (Fitt 1984; Fontellas-Brandalha and Zucoloto, 2004; Van mele *et al.,* 2009; Joachim-Bravo *et al.*, 2001) while the ovipositing preference is influenced by odor, color and shape of host fruit (Rauf *et al.*, 2013; Fitt, 1986; Ren Li-Li1 *et al.*, 2008).

The whole generation of a fruit fly takes around 37 days depending on environmental factors mainly temperature (Fletcher, 1987; Rwomushana *et al.*, 2008). The adult fruit flies are sexually mature and start to mate 4-10days after emergence from the pupae. They have a pre-oviposition period of 7-8 days. The adult lays an average of 15 eggs of 2-5 mm in size per day singly or in clusters (White and Elson-Harris 1992). The egg hatches within 3-12 days into white maggots that are 7-8mm (Ekesi and Billah, 2007). The emerged larvae take a period of 7-8 days and go through three instars (White and Elson-Harris, 1992) before developing into pupae that are black, brown or white. The pupae take a period of 10 to 20 days before emerging as an adult (Billah *et al.,* 2009). The adult crawls out of the soil and tends to take advantage of cracks in hard ground (Christenson and Foote, 1960).

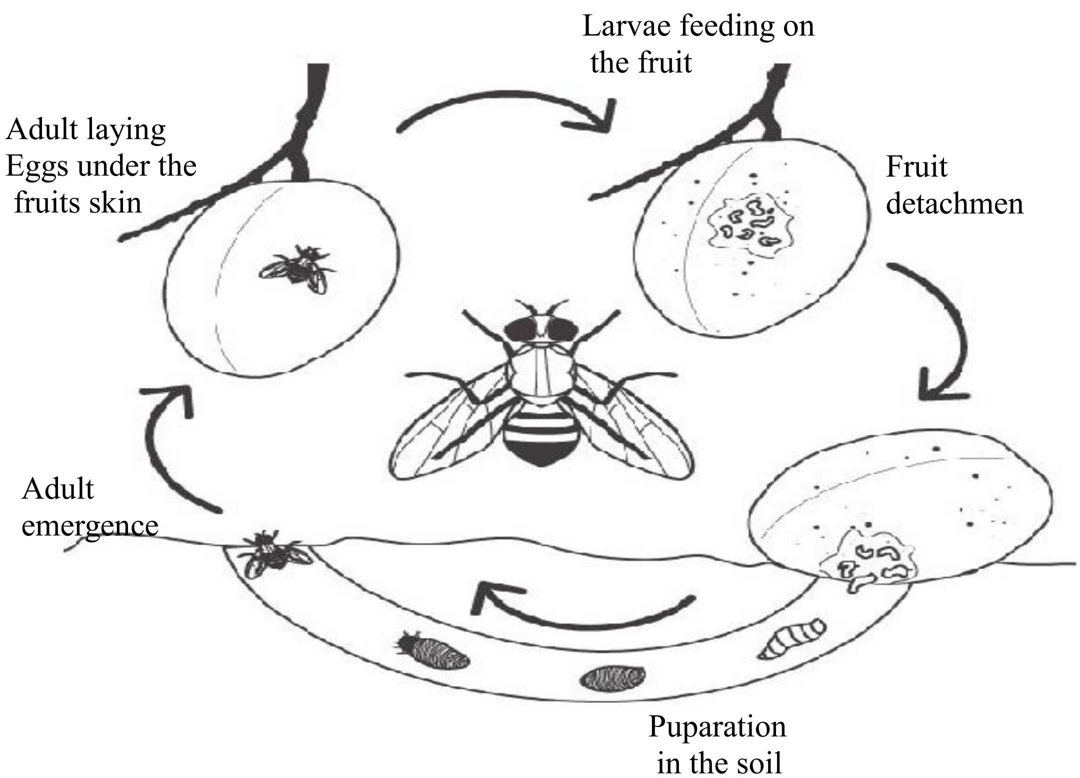

Figure 2.6. Life cycle of Tephritid fruit flies. (Source: Ekesi and Billah, 2007)

Fruit flies are polyphagous insects with a broad host range attacking fruits and vegetables (Mwatawala *et al.,* 2010). Female fruit flies choose ovipositing hosts based on the suitability of the performance of its offspring (Fontellas-Brandalha and Zucoloto, 2004; Joachim-Bravo *et al.*, 2001). Color, odor and shape of the fruit are other factors that influence ovipositing choice of most fruit fly species (Ren Li-Li *et al.*, 2008).

Oviposition patterns that are exhibited by most tephritid fruit flies involve arrival onto the fruit, head-butting to examine the fruit, aculeus insertion, egg deposition, aculeus cleaning and aculeus dragging (host marking) (Fitt, 1984). According to Rauf *et al. (2013) Bactrocera zonata* prefers guava with the highest number of healthy pupae recovered compared to citrus, chikoo, banana, ber, and apple in both a free and forced choice test that was carried out in Pakistan. *Bactrocera cucurbitae* preferred bitter gourd which had the highest pupal recovery with more than 80% adult recovery when given a host selection of bitter gourd, brinjal, muskmelon and pumpkin in an experiment (Sarwar *et al.,* 2013). *Dacus tryoni* and *Dacus jarvisi* preferred to lay eggs in fruit hosts that already had larvae in them because of the chemicals that were being released due to decomposition that accompanied larvae feeding (Fitt, 1984). Studies have also shown mangoes and bananas as most preferred hosts for *Bactrocera invadens* (Rwomushana and Ekesi, 2008).

Hence, it is necessary to identify in the laboratory the most preferred host by different species to assist in developing both a successful diet for laboratory rearing and a good pest management program for cucurbit.

2. 6 Losses caused by Tephritid fruit flies.

Tephritid-fruit flies are key pests of economic importance in the production of horticultural crops (Deguine *et al.,* 2012). This is because they attack the harvestable parts i.e. the fruits which are irreplacable. A few have been reported to cause damage on vegetables too (Ryckewaert *et al.*, 2010). The tephritid fruit flies are thus considered the most destructive worldwide (White and Elson-Harris, 1992), because the plants cannot compensate the losses as the damage is irreversible. These losses are assessed as the percentage of the infestation of mature fruits (Stonehouse *et al.,* 2007).

Fruit flies (Diptera: Tephritidae) are recorded to cause both direct and indirect losses. Direct damage is associated with female oviposition punctures that consequently cause entry of opportunistic diseases, while feeding of the larvae on fruit tissue leads to premature ripening and falling of fruits and rotting (Ekesi and Mohamed, 2011). Indirect losses are due to quarantine measures imposed by importing countries to prevent an introduction of the fruit fly into recipient countries (Mugure, 2012). These two types of damages limit the economic value of the horticultural crops and reduce crop quality and quantity for use in domestic and export markets (Price, 1999).

Tephritid fruit-flies are a global challenge to the horticultural industry as a result of the economic losses incurred. According to Barnes and Venter (2006) *Ceratitis rosa* (Karsch) and *Ceratitis cosyra* (Walker) causes losses in deciduous industry of approximately US $3Million per annum in Southern Africa while *Bactrocera* was documented to have caused losses of approximately US $2Million in Pakistan (Stonehouse *et al.,* 1998). In Benin, fruit flies (Tephritidae) cause more damage to the mango industry and other horticultural crops than any other pest (Vayssières *et al.,* 2009; Ayssieres *et al.,* 2008). Mango export in Cote d'Ivoire has been threatened by two species of fruit flies (Tephritidae) that is *Bactocera invadens* and *Dacus ciliatus* (Hala *et al.,* 2006). A field experiment by Sapkota (2010) showed that 40% of the damage on squash was caused by cucurbit fruit fly while the other losses were due to hailstorms, abnormal growth and caterpillars.

Due to the infestation rate ranging 21.5 – 71.5%, *B. cucurbitae* has caused more than 30 % economic losses on sweet gourd and ridge gourd in Bangladesh (Amin *et al.,* 2011). Tephritidae are hence disastrous pests on horticultural crops which are mainly introduced into new places by human activities particularly trade (Qin *et al.,* 2015). Therefore, more research is required to understand these fruit flies in order to manage them and bring them to acceptable injury levels.

2.7 Management of fruit flies (Tephritidae)

Fruit flies have successfully been managed using different techniques grouped into cultural, legislative control, biological controls and chemical methods. Field sanitation (Klungness *et al.*, 2005), early harvesting and bagging of fruits (FAO, 2012), are the primary cultural and mechanical methods used in the management of tephritid fruit-flies. These cultural methods are commonly used because they reduce losses incurred due to fruit flies (Tephritidae) (Ekesi and Billah, 2007).

Use of biological methods such as the use of natural enemies or predators in the management of tephritid fruit-flies has proven to be successful (Purcell, 1998; Ovruski *et al.*, 2000). Some of the biological agents used include parasitoids, predators, entomopathogens, pheromones, and bait. Parasitoids are insects whose larvae develop by feeding on the bodies of other insects resulting to the death of the target insect pest (Godfray, 1994). *Fopius arisanus,* as a parasitoid, has been used successfully in the management of *Ceratitis capitata*, and *Bactocera dorsalis* because it prevents the development of eggs into larvae of the fruit fly species named (Vargas *et al.*, 2001). Predators which are beneficial insects that feed on other insects have also been used in fruit fly management For example, the African weaver ant (*Oecophylla longinoda*) has been shown to significantly reduce the population of fruit flies (Van Mele *et al.*, 2007). Entomopathagenic fungi, that is, microbes that act as parasites of insects by killing or disabling them are also satisfactory biological controls of fruit flies (Mar and Lumyong, 2012). They reduce the fecundity and fertility of the adults (Ekesi *et al.*, 2007). The spores of the fungi (*Metarhizium anisopliae*) are mixed with fruit fly attractants that are either sprayed or inoculated to target the pupating larvae in the soil (Ekesi *et al.,* 2007). Pheromones are chemical substances that are released by insects on the outside of the body that affect the behavior and physiology of members of its species (Wyatt, 2003). Tephritid fruit flies are some of the insects that have been well managed using pheromones such as male and female attractants (Ekesi and Billah, 2007). Cuelure which is a male attractant is recorded to decrease fruit fly infestation on sweet gourd farms by over 40% when sprayed on sweet gourd (Nasirrudin *et al.,* 2002). Use of bait traps, which are insect traps made of pheromones and attractants has also proved effective in the management of tephritid fruit flies. For example, GF- 120 NF Naturalyte that contains Spinosad A and D, Propylene glycol is a fruit fly bait that works by attracting female fruit flies that have been deprived proteins, reduced fruit fly population to 0 % alive, when sprayed on cucumber farms (Prokopy *et al.,* 2003).

Use of chemical insecticide has been reported to effectively reduce fruit fly infestation, however, their frequent and repeated use has led to the development of resistance towards most of these insecticides (Ryckewaert *et al.,* 2010). *Bactocera cucurbitae* has shown the most increased resistance to the used insecticides (Sapkota, 2010). Malathion mixed with fruit fly bait spray suppresses *Ceratitis capitata* (Peck and McQuate, 2000). But it has been associated with detrimental side effects on the natural enemies (Urbaneja *et al.,* 2009).

Sterilization is also among the most used management methods in reducing losses due to tephritid fruit flies (Shelly *et al.,* 2007). The Sterile insect technique (SIT) involves the release of sterile males to mate with the females, with the hope of producing no progeny and hence reduced population of the fruit fly pests. Use of Sterile insect technique has been successfully employed in the management of *Ceratitis capitata* in Hawaii (Neto *et al.,* 2012; Shelly *et al.,* 2007). It is important to develop long-term prevention and management methods of this pest through integrated pest management because of the quarantine and resistance nature of most tephritid fruit flies.

2.8 Rearing Tephritid fruit flies in the laboratory

Successful rearing of fruit flies in the laboratory has been documented using either host plant or an artificial diet given either in liquid or solid form (Chang *et al.,* 2007; Hanife, 2008; Ekesi *et al.,* 2007). All these artificial diets developed have bulking agents, acidifying agents, and preservatives and water in common as observed in the various diets developed.

Artificial diets for different tephritid fruit-flies have been prepared and have been used successfully in rearing. For example, carrot yeast based artificial diet which is a mixture of brewer's yeast, Methyl p-hydroxybenzoate, Sugar, Citric acid, carrot powder and water is among the diets that have been developed in the mass rearing of *Bactrocera invadens* and some *Ceratitis* species (Ekesi and Mohamed, 2011). Other successful diets developed are Meridia diet that uses corncob as the bulking agent in rearing *Ceratitis capitata* (Chang *et al.,* 2007), LBI2240: FNI LS65 yeast (3:1) liquid diet that is used in rearing *Bactrocera dorsali* (Ling *et al.*, 2006) and mill feed diet used in rearing *Bactrocera cucurbitae* (Chang *et al.*, 2004). Most of the diets take into account the species preferred host and add it to the diet. *Bactocera oleae* used in the Sterile insect technique experiment, has also been reared for many generations using a modified agar-based diet which is a mixture of soy hydrolysate, yeast, sugar, casein, wheat germ, microcellulose and agar (Hanife, 2008). However, in the absence of artificial diets, tephritid fruit flies can be reared on a preferred host plant. The adult, however, is kept on a solid diet of 3:1 Hydrolysate enzyme and sugar, and water (Fletcher, 1987).

CHAPTER THREE

DIVERSITY OF TEPHRITID FRUIT FLY SPECIES INFESTING CUCURBIT AND OTHER MAJOR HORTICULTURAL CROPS IN COASTAL KENYA

3.1 Abstract

Tephritid fruit flies are categorized as major pests of horticultural crops globally causing yield losses varying from 30-80%. Tephritids have been identified on farms using traps in Kenya, but there is no record on the infestation indices of tephritid fruit flies infesting Cucurbit and the extent of damage caused. A field surveillance study was conducted on cucurbit and other major horticultural crops grown in Coastal Kenya from November 2013 to April 2014 to determine the diversity and relative abundance of tephritid fruit flies on these crops. Samples were randomly collected in various parts of the Coast region. Cucurbit crops including bittergourd, cucumber, zucchini, butternut, pumpkin, luffa, calabash, sweet melon, stripped watermelon and green watermelon were sampled together with other major crops from the family *Solanaceae*, *Anacardaciae, Rutaceae* and *Myrtaceae*. *Bactocera cucurbitae, Dacus bivitattus, D. ciliatus, D. vertebratus*, *B. invadens* and *Ceratitis cosyra* were the major tephritid fruit fly species detected. However, *B. cucurbitae*, *D. bivitattus*, *D. ciliatus*, and *D. vertebratus* were the important species causing significant yield losses in cucurbit farms. Among the cucurbit's sampled bittergourd (*Momordica charantia*) and watermelon (*Citrullus lanatus*) had the highest infestation level of up to 67%. *Bactrocera cucurbitae* and *D. bivitattus* showed highest percent infestation indices on bittergourd (*Momordica charantia*) respectively while *D. vertebratus* and *D. ciliatus* showed highest infestation indices on watermelon (*Citrullus lanatus*) and pumpkin (*Cucurbita maxima*) respectively. *Bactocera invadens* and *C. cosyra* showed little or no infestation on cucurbit but they showed significant infestation on *Solanaceae*, *Anacardaciae*, *Rutaceae* and *Myrtaceae*. These findings indicate that fruit flies are a problem in horticultural production in coastal Kenya

and that they have host preferences. This information can be used to develop management programmes for tephritid fruit flies taking into consideration their host plants and phytophagous nature to improve horticultural production.

3. 2 Introduction

Horticulture is a significant agricultural subsector in Kenya. It plays an important role in the fight against food and job insecurity. It employs 80% of the population and contributes 50% of the country's foreign exchange (Irungu, 2011). This subsector, however, faces major challenges of both biotic and abiotic factors (Ndung'u 2010). Cucurbit crops are among the major crops cultivated locally in Kenya because of the nutritional, economic and medicinal values which they have (Njoroge, 2012). Pests and diseases have been consistently classified as number one constraints of cucurbit farming because of the high crop yield losses of greater than 25% and the high cost implication incurred during their management (Davis *et al.,* 2008; Sapkota, 2010). Tephritid fruit flies are ranked among the top most notorious pests of cucurbit (Dhillon *et al.,* 2005; Kumar *et al.,* 2006; Badii *et al.,* 2015).

Tephritid fruit flies are recorded as the most devastating pests of the horticultural industry (Ekesi, 2010). This is because they contribute to high crop yield losses which decrease value and the marketability of horticultural crops (FAO, 2012; Ekesi, 2010; Meats *et al.,* 2012; White and Elson-Harris, 1992). In Africa, the genera *Bactrocera*, *Ceratitis* and *Dacus* (Ekesi and Billah, 2007) are the primary fruit fly species that cause the highest percentage of losses varying from 30-80% in the horticultural industry (Jose *et al.,* 2013; Badii *et al.*, 2015). Most of the research has, however, focused considerably on *Bactrocera invadens*, *Ceratitis cosyra* and *Ceratitis Capitata* (Ekesi *et al.*, 2009; Wharton *et al.*, 2000; Rwomushana and Ekesi, 2008) but on-farm traps have identified and associated *Dacus* species and other *Bactrocera* species as significant pests of horticultural crops (Ekesi and Billah, 2007).

Bactrocera cucurbitae and *Dacus* species in most regions of the world are associated with >50% yield losses in cucurbit farms (Kumar *et al.*, 2006; Dhillon *et al.*, 2005; Sapkota, 2010). *Bactocera cucurbitae* (melon fly) is a serious pest of the horticultural industry and is considered native to India (Sapkota, 2010). It was introduced in some African, Asian, N. American and Oceania countries through human activities such as trade (Weems *et al.,* 2012). *Dacus* species are also of Asian origin and are reported to have been introduced to other regions of the world through human activities (White and Elson-Harris, 1992; Weems, 2015).

In Kenya, these species have been associated with major losses in cucurbit farms. However, the losses have not yet been quantified. Determining the infestation index of *B. cucurbitae* and *Dacus* species in cucurbit farms will facilitate the evaluation of the level of economic losses that these species cause. These would be used to rationalize the need for further research on these fruit fly species that would enable development of robust pest management strategies to improve cucurbit production and reduce the losses incurred in horticultural farms. The aim of the study was to determine the diversity and relative abundance of tephritid fruit flies on cucurbit crops.

3.3 Materials and methods

Sampling site description

Coastal Kenya has a tropical climate with rainfall and temperatures higher throughout the year. The temperatures in the Coast range from ±28-33° C while receiving an average of 1000mm rainfall annually according to the Kenyan meteorological department 2016 (www.meteo.go.ke).

3.3.1 Sampling of Tephritid fruit flies in Coastal Kenya

Infested fruits were randomly sampled from the Coastal Kenya between November 2013 and April 2014. The sampling regions were Kilifi, Mombasa, Shimba hills, Muhaka, Kwale and Kibarani and were selected based on information provided by the Coast Ministry of Agriculture on cucurbit farming and also based on the availability and diversity of cucurbit crops. In the each region, 10 farms were randomly identified, and the number of *Cucurbits* collected was based on the availability on each farm. The fruits sampled included those that showed visible symptoms of fruit fly damage such as fruit fly punctures and presence of fruit fly larvae that were either still attached to the plant or had fallen on the ground. For each farm large quantity collections were attempted with a minimum of at least three kilogram of fruit per fruit species. However, in some cases the sample size could not be maintained due to availability.

3. 3.2 Determination of infestation of Tephritid fruit fly infestation

Fruit sampling and processing procedure was established using the methodology described by Ekesi and Billah (2007). Different cucurbit such as bittergourd, cucumber, zucchini, butternut, pumpkin, luffa, calabash, sweet melon, stripped watermelon and green watermelon together with other major crops from the family *Solanaceae*, *Anacardaciae*, *Rutaceae* and *Myrtaceae* were randomly collected from various farms in Coastal Kenya. The number of fruits collected depended on availability and at least three Kilogram of fruit was collected from each farm sampled. Samples collected were then placed in well-labeled polyethylene bags with small holes to keep off moisture and to avoid suffocating the larvae. Global position system (GPS) coordinates and field photographs were taken for field referencing. The samples were then transported to the laboratory under room temperature.

In the laboratory, various fruit species were weighed and placed in different containers. The fruit species were divided into three groups that were observed as replicates and incubated separately to determine fruit infestation indices per Kilogram of fruit (Rwomushana and Ekesi, 2008). Later the three replicates of each fruit species sampled were separately placed in dish drying racks that were placed on fruit incubation square boxes that had a stand and sand at the bottom for pupation.

Smaller fruits were placed in two liters, while the larger fruits were placed in four liters containers with ellipsoid holes equivalent to 0.5 and 2.5 cm at the base. The two liter containers were then placed on four liter containers and covered with tops that had the middle section cut and replaced with a net for aeration. The ellipsoid holes were to allow mature larvae to fall into the larger container for pupation. Samples were incubated, and pupae collected after seven days. Fruits that had not properly decayed after the five days were dissected to check whether there were any larvae as described by Ekesi and Billah (2007). Collected pupae were counted, and the numbers recorded. Pupae were then placed on Perspex cages of 15 by 15 by 15cm with a wet cotton wool and a petri dish with sugar and enzymatic yeast hydrolysate ultrapure in a 3:1 ratio for feeding emerging adults. Emerging adults were allowed seven days to enable full body coloration and maturation for identification. The adults were knocked down by freezing for five minutes in a -20 freezer to allow morphological identification under the microscope without killing the insects as they were used to start a colony. The identification of the adults was based on morphological characteristics such as the markings on the wings, leg coloration, abdominal markings and coloration of lateral markings on the thorax using a dichotomous key (White, 2006; White and Elson-Harris, 1994; Ekesi and Billah, 2007).

Adults were later separated based on species and placed in different Perspex cages of 30cm by 30cm by 30cm. The various adult species numbers were compared to the weight of samples collected to determine the infestation indices as per Cowley *et al.*, (1992) that is ratio of number of adults/Kilogram of fruit samples. Relative Abundance Index (RAI) was later calculated as per Segura *et al.,* (2006) using the formula $RAI_{B.c=}$ B.c/ (B.c+D.b+D.c+D.v) where each initial represent one of the fruit fly species identified to determine prevalence of the tephritid fruit flies identified on the various crops sampled.

3. 3. 3 Identification of Tephritid fruit flies

Adult fruit flies were identified morphologically under a dissecting microscope using dichotomous keys under a magnification of 20X (White, 2006; White and Elson-Harris, 1994; Ekesi and Billah, 2007). The key features used for identification were tergite separation, scutellum coloration, color of the lateral and median stripes on the thorax, color of femora and the tibia, and wing coloration and the characteristic of the apical spot (White, 2006; White and Elson-Harris, 1992).

3. 4 Data analysis

The infestation indices of the various tephritid fruit fly species in the different horticultural crops were determined using Cowley *et al.,* (1992) method that is calculated as the ratio of the number of flies/Kilogram of fruit sampled. Relative Abundance Index (RAI) was further calculated to determine the relationship of *B. cucurbitae*, *D. ciliatus*, *D. bivitattus* and *D. vertebratus* in the various host plants using Segura *et al.,* (2006) method that is calculated as $RAI_{B.c=}$ B.c/ (B.c+D.b+D.c+D.v) where each initial represent a fruit fly species. Relative Abundance Index (RAI) values from the different host plants and fruit fly species were compared using a non-parametric analysis of variance (ANOVA) (Kruskal-Wallis test) using R. When the analysis showed significant difference, a non-parametric multiple comparison Dunn test was performed (Segura *et al.*, 2006).

3. 5 Results

3. 5.1 Infestation indices of insects which were identified during the field sampling

A total of 17 major crops were sampled in Coastal Kenya and among the sampled crops 10 were cucurbit. *Bactrocera cucurbitae*, *D. bivitattus*, *D. ciliatus*, and *D. vertebratus* listed in figure 3.1. were the major tephritid species identified as pests of cucurbit during the surveillance study.

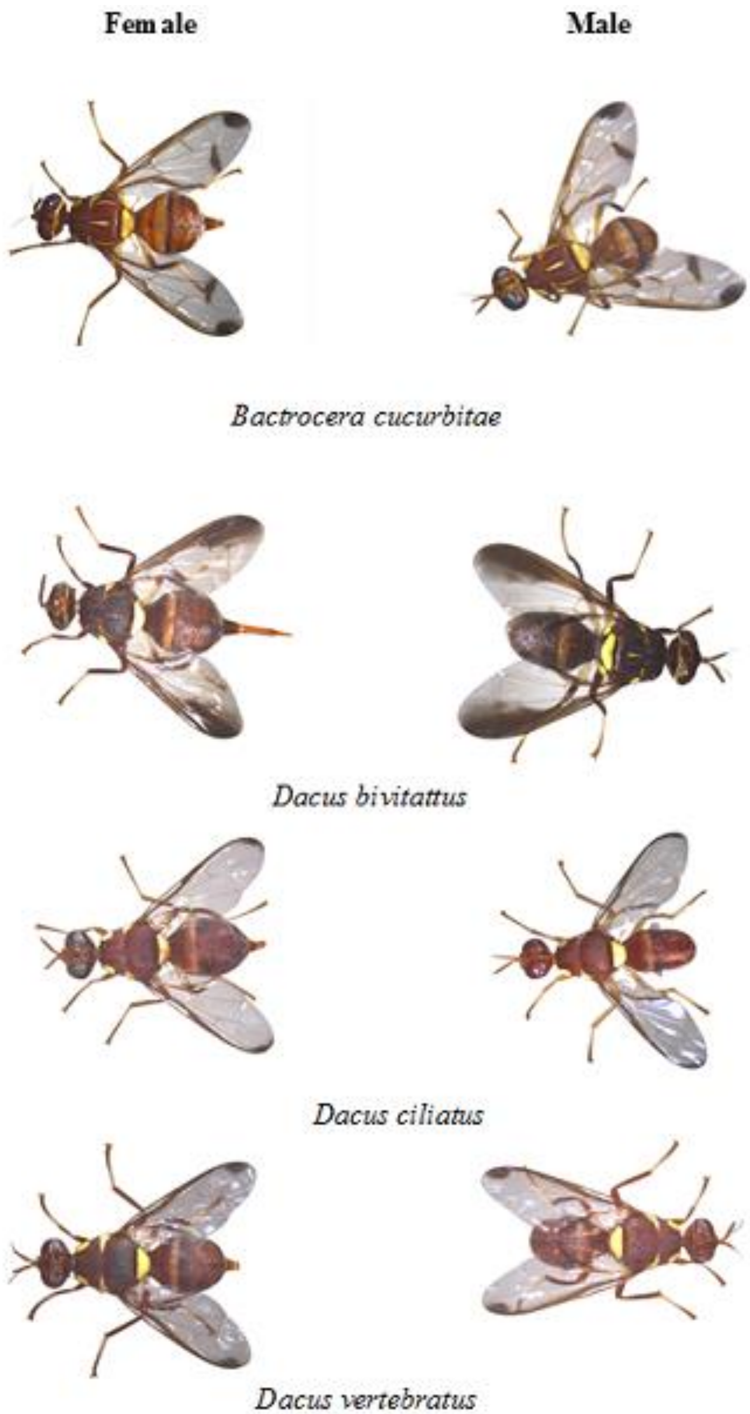

Figure 3.1. Tephritid fruit flies identified from the cucurbit sampled in Coastal Kenya

The sampled cucurbit were bitter gourd, cucumber, melon, pumpkin, courgettes, stripped watermelon, sweet melon, calabash, and luffa. Bitter gourd had the highest of tephritid fruit fly infestation (Table 3.1) with *B. cucurbitae* and *D. bivitattus* as the dominant species. Stripped watermelon had the second highest percentage infestation with *B. cucurbitae* and *D. vertebratus* as the dominant species in this fruit (Table 3.1). Cucumber and Pumpkin followed with the highest infestation indices with *D. ciliatus* as the prevalent species (Table 3.1). However, there was no significant difference in the infestation index of the various tephritid fruit flies on each crop sampled (Table 3.1). There was also no significant difference in the diversity of tephritid fruit flies in the various crops sampled (Table 3.1).

Bactrocera invadens and *C. cosyra* were also recorded from the various cucurbit but in negligible numbers. *Bactocera invadens* showed the highest infestation indicex of 12.7 on butternut and the lowest on sweetmelon, luffa and Zucchini (Table 3.1). *Ceratitis cosyra* showed an infestation indices of greater than 1.2 on all the sampled cucurbit. These two fruit fly species showed a significant difference in the infestation index of the various crops sampled (Table 3.1).

The other horticultural crops sampled were pepper, tomato, eggplant (*Solanaceae*), mango **(**_Anacardiaceae_), sweet orange, tangerine (*Rutaceae*) and guava (*Myrtaceae*). Among these fruits tomato had the highest infestation of fruit fly (Table 3.1). Suprisingly, *B. cucurbitae* had the highest infestation index on tomato fruits of 60.2% (Table 3.1). *Dacus ciliatus* also showed prevalence on mango and guava with high infestation index (Table 3.1). Among all the fruits sampled eggplant (3.2%) and sweet melon (2.1) had the lowest infestation indices of fruit flies, respectively.

Table 2.1. Infestation indices of *Bactrocera cucurbitae* and *Dacus* species on cucurbit and other horticultural crops in coastal Kenya

Fruit Species	No. of fruits	Fruit weight (Kg)	Infestation (%)	No. flies/Kg fruit						
				B.c	D.c	D.b	D.v	B.i	C.c	**Mean**
Bitter gourd (*Momordica charantia*) (L)	132	29.7	66.8	16.2	3.3	12.3	2.1	2.6	0.0	**6.1**
Cucumber (*Cucumis sativus*) (L)	125	31.3	30.2	3.7	5.2	3.4	0.0	4.8	0.0	**2.9**
Green melon (*Cucumis melo*) (L)	113	14.2	10.4	1.5	0.0	1.2	0.0	1.1	0.2	**0.7**
Pumpkin (*Cucurbita maxima*) (Duch)	73	115.3	28.8	4.2	6.8	1.1	4.7	0.6	0.7	**3.0**
Zucchini (*Cucurbita pepo*) (L.)	102	30.7	12.8	1.3	3.7	3.2	2.1	0.0	0.0	**17**
Butternut (*Cucurbita moschata*)	134	12.5	16.2	3.1	1.3	10.4	3.2	12.7	1.2	**5.3**
Stripped melon (*Citrullus lanatus*) (T.) Mats	429	119.4	60.6	13.3	1.8	0.0	14.3	1.1	0.0	**5.1**
Calabash (*Lagenaria siceraria*) (M.) Standl	111	60.2	10.2	1.5	0.0	2.5	0.0	0.3	1.2	**0.9**
Luffa (*Luffa cylindrica*) (L.) Roen	142	21.7	6.9	1.3	1.4	0.0	0.0	0.0	0.0	**0.5**
Sweetmelon (*Cucumis melo*) var. conomon)	102	66.2	3.8	0.8	0.0	0.0	1.8	0.0	0.0	**0.4**
Pepper (*Capsicum frutescens*) (L.)	789	1.3	6.2	0.6	2.6	0.8	0.4	3.7	5.2	**2.2**
Tomato (*Lycopersicum esculentum*)	131	7.7	60.2	12.1	1.4	0.0	0.0	1.8	1.6	**2.8**
Eggplant (*Solanum* melongena)	76	3.5	2.1	1.1	2.8	1.1	0.0	0.0	0.0	**0.8**
Mango (*Mangifera indica*)	109	31.6	52.4	0.5	24.1	0.0	0.0	121.0	0.4	**24.3**
Sweet orange (*Citrus sinensis*)	93	7.4	26.6	0.5	2.1	0.0	0.0	1.8	2.3	**1.1**
Tangerine (*Citrus reticulata*)	40	2.8	11.8	0.2	1.1	0.0	0.4	2.1	2.7	**1.1**
Guava (*Psidium guajava*)	42	3.8	20.4	0.4	14.7	1.7	0.0	7.8	1.5	**4.4**
Mean	**137.6**	**32**	**25.1**	**3.7**	**4.1**	**2.2**	**1.6**	**9.5**	**1.0**	
C.V (%)	**-**	**-**	**-**	**0.4**	**1.0**	**0.6**	**0.4**	**0.6**	**0.2**	
LSD (p<0.05)	-	**-**	**-**	**3.7**	**4.1**	**2.2**	**1.6**	**9.5**	**1.0**	

B.c- *B. cucurbitae*; D.b-*D. bivitattus*; D.c-*D. ciliatus*; D.v-*D. vertebratus*; B.i- *B. cucurbitae*; C.c- *C.cosyra*. There was significance difference in the number of flies per kilogram of fruit (F=1.049; df=1.042; p=0.397).

(C.V calculated by first transforming the data to log $_{10)}$

3.5.2 Relative abundance index of the different tephritid fruit flies

The relative abundance index (RAI) was divided into four categories; complete absence of a particular species was represented by 0; high abundance of a particular species was represented by values greater than 0.5; equal abundance of all species in a particular host was signified by 0.25 and low abundance of a particular species was signified by values lower than 0.25. *Bactrocera cucurbitae* was more dominant than the other fruit fly species on green melon and was least dominant on zucchini among the sampled cucurbit (Table 3.2). Among the other non- cucurbit crops sampled *B. cucurbitae* was most dominant on tomato compared to the other fruit fly species (Table. 3.2). *Dacus ciliatus* was dominant on cucumber and was completely absent on green melon and the calabash (Table 3.2). Among the non-cucurbit this species, *D. ciliatus* was dominant on mango, sweet orange and guava (Table. 3.2). *Dacus bivitattus* was the most abundant tephritid fruit flies on calabash and butternut, respectively (Table 3.2). *Dacus vertebratus* was most abundant on watermelon and sweet melon, respectively. No dominance was observed on the non-cucurbit (Table. 3.2). *Bactrocera cucurbitae*, *D. bivitattus* and *D. ciliatus* showed no significant difference in the infestation of the different cucurbit and horticultural crops but *D. vertebratus* showed a significant difference with the fruit flies in infesting the different sampled crops (Table 3.2). The species had the least RAI indicating almost absent/absence of the species in the area or farms. Table 3.3 showed that there was significant difference in the Relative abundance index of *Cucurbitaceae*, *Solanaceae*, *Anacardaceae*, *Rutaceae*, and *Myrtaceae*. Table 3.4 showed that there was significant difference in the RAI values of the four fruit fly species on the various crop families.

Table 3.2. Relative abundance index values of tephritid fruit flies in different host plants

Fruit Species	Sampled fruits	Fruit Infestation %	Relative Abundance Index				
			RAI B. c	RAI D. c	RAI D. b	RAI D. v	**RAI Mean**
Bitter gourd (*Momordica charantia*) (L)	132	66.8	0.478	0.097	0.363	0.062	**0.25**
Cucumber (*Cucumis sativus*) (L)	125	30.2	0.301	0.423	0.276	0.000	**0.25**
Green melon (*Cucumis melo*) (L)	113	10.4	0.556	0.000	0.444	0.000	**0.25**
Pumpkin (*Cucurbita maxima*) (Duch)	48	28.8	0.328	0.297	0.086	0.289	**0.25**
Zucchini (*Cucurbita pepo*) (L.)	102	12.8	0.126	0.359	0.311	0.204	**0.25**
Butternut (*Cucurbita moschata*)	134	16.2	0.172	0.072	0.578	0.178	**0.25**
Stripped melon (*Citrullus lanatus*) (T.) Mats	51	60.6	0.452	0.061	0.000	0.486	**0.25**
Calabash (*Lagenaria siceraria*) (M.) Standl	111	10.2	0.375	0.000	0.625	0.000	**0.25**
Luffa (*Luffa cylindrica*) (L.) Roen	142	6.9	0.481	0.519	0.000	0.000	**0.25**
Sweetmelon (*Cucumis melo*) var. conomon)	102	3.8	0.308	0.000	0.000	0.692	**0.25**
Pepper (*Capsicum frutescens*) (L.)	789	6.2	0.136	0.591	0.182	0.091	**0.25**
Tomato (*Lycopersicum esculentum*)	131	60.2	0.896	0.104	0.000	0.000	**0.25**
Eggplant (*Solanum* melongena)	76	2.1	0.220	0.560	0.220	0.000	**0.25**
Mango (*Mangifera indica*)	109	52.4	0.020	0.980	0.000	0.000	**0.25**
Sweet orange (*Citrus sinensis*)	93	26.6	0.192	0.808	0.000	0.000	**0.25**
Tangerine (*Citrus reticulata*)	40	11.8	0.118	0.647	0.000	0.235	**025**
Guava (*Psidium guajava*)	42	20.4	0.024	0.875	0.101	0.000	**0.25**
Mean	**137.6**	**25.1**	**0.320**	**0.187**	**0.379**	**0.113**	
C.V (%)	**124.2**	**109.6**	**-1.540**	**-0.970**	**0.630**	**-0.950**	
LSD ($p<0.05$)	**-**	**-**	**0.320**	**0.187**	**0.379**	**0.113**	

The RAI values were assigned to the four categories: complete absence of a particular species (RAI=0) absence; high abundance of a particular species (RAI>0.5); equal abundance of all species in a particular host (RAI=0.25) and low abundance of a particular species (RAI<0.25). (B.c- *B. cucurbitae*; D.b-*D. bivitattus*; D.c-*D. ciliatus*; D.v-*D. vertebratus*). LSD test F=4.11; df= 3, 64; p=0.01.

Table 3.3. Relative abundance index values of the various host plant families

Host family	RAI	Q25	Q75	N
Cucurbitaceae	0.24	0.046	0.387	10
Solanaceae	0.159	0.0682	0.305	3
Anacardaceae	0.01	0	0.26	1
Rutaceae	0.155	0	0.338	2
Myrtaceae	0.0625	0.018	0.295	1

Kruskal-Wallis X^2=0.947; df= 4; p=0.92. There was significant difference in the RAI values of the different families. The data range between Q25 and Q75 shows that there is variation in the RAI values of the various families. Q25= the lower quartile; Q75= the upper quartile; N= the total number of crops in a family.

Table 3.4. Mean separation of fruit fly RAI values of all host plants

Fruit flies	RAI
B. cucurbitae	0.301[a]
D. bivitattus	0.101[ab]
D. ciliatus	0.359[a]
D. vertebratus	0[b]

Kruskal-Wallis X^2=12.5819; df= 3; p=0.005 Medians followed by different letters differed statistically (P<0.05; multiple comparison Dunn's test). There was a significance difference in the relative abundance index of the tephritid fruit flies in the various crops.

3.6 Discussion

The results presented show that among the tephritid fruit flies infesting the cucurbit, *Dacus* genus, was represented by *D. ciliatus*, *D. bivitattus*, and *D. vertebratus*, while the *Bactrocera* genus was represented by *B. cucurbitae*. These findings compare with the documented evidence of traps catches of these fruit fly species using food baits by Ekesi and Billah (2007). However, this is the first major report of fruit infestation by these fruit flies on cucurbit at the Kenyan Coast. Among the various cucurbit sampled, bittergourd and stripped melons suffered the highest infestation by fruit flies. *Bactrocera cucurbitae* emerged the top fruit fly infesting cucurbit with the highest relative abundance index value on stripped melon, bittergourd and pumpkin followed by the three *Dacus* species i.e. *D. ciliatus*, *D. bivitattus* and *D. vertebratus.* Melon fly (*Bactrocera cucurbitae*) has been reported to be an important pest of cucurbit and depending on the season, the pest can inflict 30 to 100% loss on the crop (Dhillon *et al.,* 2005; Vayssières *et al.,* 2006). It is reported that bittergourd, watermelon, and pumpkin are among the preferred host plants of *B. cucurbitae* in the wild (Lanjar *et al.,* 2013; Amin *et al.*, 2011; Vayssières *et al.,* 2007). This would hence, explain the high infestation percent and dominance of *B. cucurbitae* on these crops during the sampling study. In addition, the results give a first record of *B. cucurbitae* heavily infesting tomatoes and this is something that should be followed up to confirm its distribution as a tomato pest in the country. *Bactrocera cucurbitae* was also observed to infest other crops from *Solanaceae*, *Rutaceae, Myrtaceae* and *Anarcadiaceae* families. However, the infestation rate was not as high as that in the *Cucurbitaceae* family. Regions such as Tanzania and West Africa have reported *B. cucurbitae* as a significant pest of these crop families (Vayssières *et al.,* 2007; Mwatawala *et al.,* 2010; White and Wang, 2009; Ramadan and Messing, 2003).

Dacus genus followed the infestation of *Bactrocera* genus on cucurbit. *Dacus* species have been recorded as a significant pest of cucurbit (White and Elson-Harris, 1992). *Dacus ciliatus* in this study, the result indicated that cucumber and pumpkin were its preferred hosts respectively. *Dacus ciliatus* (lesser pumpkin fly) is documented as an economic pest of cucurbit because of the enormous yield losses which it causes on cucurbit farms (White and Elson-Harris 1992; Kumar *et al.,* 2006; Alagarmalai *et al.,* 2009; Badii *et al.,* 2015). Cucumber, courgettes, and luffa were recorded to support the development of *D. ciliatus* compared to the other three species *B. cucurbitae*, *D. bivitattus* and *D. vertevratus* as they had the highest RAI value of *D. ciliatus*. An earlier study had shown that cucumber, courgettes and pumpkin are preferred and suitable hosts of *D. ciliatus* because they support successful development of immature stages of this species (Vayssières *et al.,* 2008). Other studies in different regions of the world have shown that the two host plants are listed among the preferred hosts of *D. ciliatus* (Badii *et al.,* 2015; Weems, 2012). In addition, *D. ciliatus* with its high RAI on mango, citrus species and guava, pinpoints its importance as a pest of these crops as well and may need further follow up to confirm distribution and loss quantification in the country. Among the *Dacus* sp. identified, *D. ciliatus* was observed to be a significant pest of the other crops from the families *Solanaceae*, *Rutaceae*, *Myrtaceae* and *Anarcadiaceae* as compared to *D. bivitattus* and *D. ciliatus*. These families were observed to support the development of *D. ciliatus* which had the highest RAI values more than the other two *Dacus* sp. and *B. cucurbitae*. *Dacus ciliatus* has previously been listed as a significant pest of these families in Africa (White and Elson-Harris, 1992; Ekesi and Billah, 2007).

Greater pumpkin fly (*Dacus bivittatus*) showed high preference on butternut and bitter gourd with infestation percent of 10.4 and 12.3 respectively. The fruit fly species had the highest RAI values on these crops. *Dacus bivitattus* is categorized as an economic principal pest of cucurbit listing bittergourd, butternut and calabash as among the preferred hosts of this fruit fly species in the wild (Badii *et al.,* 2015; de Meyer *et al.,* 2012). Hence, the findings are in line with the report by Badii *et al.,* (2015).

The jointed pumpkin and melon fly (*D. vertebratus*) was recorded as the least damaging species among the three *Dacus* species sampled in Coastal Kenya as it only attacked six crops of the total cucurbit sampled. However, it was observed to be a significant pest of water melon as it had its highest infestation index on this crop. Stripped melon and green melon were the suitable hosts of *D. vertebratus* with the highest relative abundance index. *Dacus vertebratus* is recorded as a pest of cucurbit's from as early as 1950's in South Africa (Naude, 1950). In Africa, surveillance studies have shown that watermelon and pumpkin farms succumb to significant yield losses due to *D. vertebratus* (Badii *et al.,* 2015).

3.7 Conclusion

The study was conducted to determine the diversity and infestation index of tephritid fruit flies infesting cucurbit in Coastal Kenya. The findings indicate that *B. cucurbitae*, *B. invadens*, *D. ciliatus*, *D. vertebratus*, *D. bivitattus*, and *C. cosyra* are the major fruit flies present in horticultural farms at the Coast of Kenya. *Bactrocera cucurbitae*, *Dacus ciliatus*, *D. bivitattus* and *D. vertebratus* are the significant fruit fly species of economic importance infesting cucurbit in coastal Kenya. The mixed infestation by differerent fruit fly species may be a contributory factor to substantial crop losses in this region. These fruit fly species also attacked other horticultural crops from the *Solanaceae*, *Rutaceae*, *Myrtaceae* and *Anarcadiaceae* families with *Dacus ciliatus* and *B. cucurbitae* being the most damaging fruiy fly species. *Bactrocera cucurbitae* highly infested tomatoes while *D. ciliatus* exhibited its polyphagous nature also infesting mango, citrus and guava. This indicates that these fruit fly species are significant pests of the horticultural industry and pest management programs covering cucurbit and other horticultural crops should be developed for these tephritid fruit flies.

CHAPTER FOUR

HOST PREFERENCE AND MASS REARING PROCEDURE FOR TEPHRITID FRUIT FLY

4.1 Abstract

Bactrocera cucurbitae and *Dacus* species are recognized as important pests of cucurbit globally. Previous host fruit assessment studies in Coastal Kenya revealed that *B. cucurbitae, Dacus bivitattus* and *Dacus ciliatus* are the primary pests of cucurbit. The objective of this study was to determine the host preference of the three fruit fly species and how they can be reared in the laboratory. Host preference studies for the three species of fruit fly species were conducted in the laboratory on butternut, watermelon, cucumber, and courgettes. This crops were selected based the fact that among all the cucurbit crops sampled, these crops are the commonly produced in Kenya in large scale. Data on pupal recovery, pupae/ml, adult emergence percent, deformity percent, sex ratio and tibia length measurements were used to assess the host preference studies. Mass rearing of the three species was tested on butternut to determine its suitability. A Mass rearing study was conducted to enable further research in the laboratory. Pupae recovered, weight of pupae, percent adult emergence, fecundity percent, egg hatch and flight ability were the parameters determined. Watermelon was the most preferred host plant by all the three fruit fly species. However, the three fruit fly species showed variation in their preference on other cucurbit. Butternut was found to be a suitable host for rearing *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* in the absence of an artificial diet. The results imply that the three tephritid fruit flies are able to choose and prefer or discriminate a host based on the suitability of the host to support its offspring. The results implied that butternut can be used in the mass rearing of *B. cucurbiatae*, *D. bivitattus* and *D. ciliatus* in the absence of an artificial diet since it supported maximum development of the three fruit fly species compared to the other fruits.

4. 2 Introduction

Fruit flies (Diptera: Tephritidae) are among the most destructive insect pests of many horticultural crops because they cause both direct and indirect economic losses (Ekesi and Mohamed, 2011; Billah *et al.,* 2006). Direct losses are due to the injuries they cause on plants and fruits that decrease crop yield, while indirect losses are due to trade restrictions imposed on quarantine pests (Sarwar *et al.,* 2013; Price, 1999). Tephritid fruit flies are divided into more than 500 genera with over 4,000 species reported across the globe (White and Elson-Harris, 1992). The genera *Bactrocera*, *Ceratitis* and *Dacus* have been highly ranked as the most notorious insect pests in Africa (Billah *et al.,* 2009; Mwatawala *et al.,* 2009; Vayssières *et al.*, 2007; Badii *et al.,* 2015; Elfékih and Haymer, 2010; Chang *et al.,* 2007; Ekesi *et al.,* 2009; Meyer, 2013).

Tephritid's are polyphagous insects in nature with a wide host range attacking both fruits and vegetables (White and Elson-Harris, 1992; Mwatawala *et al.,* 2009). In Africa, *B. cucurbitae* and *Dacus sp.* are associated with major yield losses of more than 30% incurred in horticultural crops especially from the cucurbit family (Vayssières *et al.,* 2007; Billah *et al.,* 2009; Badii *et al.*, 2015; Kumar and Verghese 2008; de Meyer *et al.,* 2012; Wiley, 2009) . Apart from cucurbits, *B. cucurbitae* also causes significant losses on other horticultural families occasionally. However, with minimal infestation rates (Mwatawala *et al.,* 2010). In West and Central Africa, *B. cucurbitae* has been reported to attack other host plants from the family *Solanaceae, Rutaceae*, *Anacardiaceae*, *Annonaceae* and *Oxalidaceae* in the absence of cucurbit's (Vayssières *et al.*, 2007). In India, *B. cucurbitae* has been associated with more than 60% losses in both cucurbit and *Solanaceae* (tomato) farms (Kapoor, 2005) .

Previous surveillance studies conducted in Kenya indicated that *B. cucurbitae*, *D. bivitattus*, *D. ciliatus*, and *D. vertebratus* were economic pests of cucurbit and other host plants from the families *Solanaceae*, *Myrtaceae*, *Rutacaceae* and *Anacardaceae* (Ekesi and Billa, 2007). Although these species are recorded to be attacking a variety of cucurbit in Kenya, there is no documentation of their host preferences.

Due to the economic importance of tephritid fruit flies, it is important to establish a successful pest management programs to reduce losses in fruits and vegetables. The development of such a management program is dependent on successful laboratory studies that rely on the establishment of a cost-effective mass rearing and quality control procedures for bioeclogical studies, classical biological control, Sterile Insect Technique, postharvest treatment and other management strategies (Ekesi *et al.,* 2003; Shelly *et al.,* 2007; Bokonon-Ganta *et al.,* 2007; Daane *et al.,* 2011). Adult tephritid fruit flies feed on foods rich in protein and sugar from bird excrement, honeydew and nectar, while larvae feed on the host fruit, however, larvae from non-frugivorous species feed on vegetables (Headrick and Goeden, 1998). Artificial diets have been developed for a variety of species including *B. invadens* (Ekesi *et al.,* 2007), *B. cucurbitae* (Chang *et al.,* 2004), and various native *Ceratitis* spp. (Chang *et al.,* 2007; Ekesi and Mohamed, 2007). Although artificial diets based on liquid diet have been established for *B. cucurbitae* (Chang *et al.,* 2004) recent observation from *icipe* showed that the Kenyan population of this insect was not amenable to rearing on artificial diet. In addition, artificial diets for species of economic importance from the *Dacus* genus such as *D. bivitattus* have not yet been developed. In the absence of an artificial diet, whole fruit rearing is advocated for tephritid fruit flies. *Dacus ciliatus* has been ranked high as a significant economic pest from the *Dacus* genus because of the potential threat it has once it is introduced to a new region and its current level of economic losses (Caceres *et al.,* 2014; White and Elson-Harris, 1992; Vayssières *et al.,* 2008). Due to its economic significance artificial rearing methods have been established using the meridic diet (Alagarmalai *et al.,* 2009). Despite successful rearing methods established in other regions of the world, a great challenge has been faced in trying to replicate this at *icipe* with the Kenyan population.

4. 3 Material and methods

4. 3.1 Determination of host preference of Tephritid fruit flies

4.3.1.1 Experimental design

The conducted experimental design for the host preference studies was developed based on the methodology described by Sarwar *et al.,* (2013). Host choice and no host choice experiments were conducted on *B. cucurbitae*, *D. ciliatus* and *D. bivitattus* at the International Center of Insect Physiology and Ecology (*icipe*). The four major cucurbit fruits which are grown in Kenya include butternut (*C. moschata*), cucumber (*C. sativus*), courgettes (*C. pepo*), and watermelon (*C. lanatus*) were used as hosts in the experiments. The mature and undamaged fruits for the tests were purchased from a local market. The experiment consisted of 12 treatments which were the interaction of the three fruit fly species with the four cucurbit and an untreated control of cucurbit. Each treatment was replicated four times and the entire experiment was repeated four times.

4.3.1.2 Rearing of Tephritid fruit flies

Tephritid fruit flies were used for the experiment were obtained from existing colonies that were established from wild fruit fly pupae collected from the cucurbit sampled in Coastal Kenya. The emerged adult fruit flies were reared on sugar and enzymatic yeast hydrolysate ultrapure in a 3:1

ratio (solid diet) and water (Ekesi and Mohamed 2007). They were reared in 35cm x 35cm x 35cm Perspex cages, and the colonies were maintained at temperatures of ± 25º C and photoperiod of 12 hours of light: 12 hours of darkness.

4.3.1.3 Determination of host preference of tephritid fruit flies using free host choice test

To determine host preference of the three fruit fly species based on free host choice, butternut cucumber, courgettes, and watermelon fruits were weighed and exposed to *B. cucurbitae*, *D. ciliatus* and *D. bivitattus*. Experiments were conducted in 35 cm by 35 cm by 35 cm Perspex cages. Each fruit was placed on the floor of the cage at a distance of 25 cm from each other. Thereafter 200 adult flies (100 males and 100 females; age10 days) were aspirated from the colony and released in the cages for a period of 24 h. Each experiment started at 0900h and was complete at 0900h the following day. The experiment had four replicates and was repeated five times.

4.3.1.4 Determination of host preference of tephritid fruit flies using no-choice test

To determine host preference of the three fruit fly species based on no-choice test, each fruit species listed above was assessed individually using the three fruit fly species under investigation. All other experimental protocol and conditions were similar to the free host choice test. The experiment also had four replicates and was repeated five times.

Later the samples from both tests were placed in the fruit incubation square boxes for pupation with sterile soil at the bottom. Samples were incubated, and pupae collected after 10 days. Fruits that had not properly decayed after the 10 days were dissected to check whether there were any larvae. The number of pupae per Kilogram of fruit was recorded to determine infestation indices which was calculated as the ratio of the number of fruit flies/Kilogram of fruit. The size of the pupae was determined by counting the number of pupae per milliliter. The pupae were later placed in petri dishes inside Perspex cages of 15cm x 15cm x 15cm. Emerging adults were kept on water and a diet containing 3:1 sugar and enzyme hydrolysate ultrapure (Ekesi and Mohamed, 2007). Adults were allowed to develop for seven days to enable full body coloration and maturation. The mature adults were then aspirated into falcon tubes with 70% ethanol to kill and preserve them. The dead fruit flies were then placed in petri dishes to count those which deformed and separate the males from the females. One hundred of the dead insects were placed under an electron microscope, the hind legs were pulled and fixed on a slide using a mixture of Distyrene, Plasticizer and Xylene (DPX). The length of the tibia was determined using LAS EZ/ leica microsystems computer software (van Casteren and Codd 2010).

4.3.1.5. Assessment of the population of fruit fly

Data collected included the total number of pupae from each host fruits, pupae per milliliter, percent adult emergence, percent sex ratio, percent adult deformity and tibia length measurements. Hind legs from 100 flies from each host (1:1 male to female) and species were removed to measure the length of tibia and the data recorded. The percentage of the number of flies that emerged was calculated from the total number of pupae collected. The sex ratio and deformity percentage were calculated from the total number of adults that emerged. Infestation indices were measured from number of flies per kilogram of fruits.

4.3.2 Determination of butternut suitability for mass rearing of Tephritid fruit flies

The rearing and quality control parameters for *B. cucurbitae, D. bivitattus,* and *D .ciliatus* on butternut (*Cucurbita moschata*) was established using the methodology described by Ekesi and Mohammed, (2011). Butternut was selected because of its availability, its lessButternut fruits were purchased from a local market in Nairobi. The fruits were weighed, thereafter washed and rinsed with 0.03% Sodium hypochlorite (NaClO). Each fruit were spiked several times using an entomological pin to facilitate oviposition by the insects. The fruits were then placed in a Perspex cage (35 x 35 x 35 cm) and 100 adult insects of each species at a ratio of 1:1 female to male were transferred into different cages. After 24 hours exposure of the butternuts to the fruit flies, the fruits were removed from the cages and placed in four liter containers, a mesh-covered top was then fitted over the containers. The four liter containers were then placed in larger containers of 10 liter with sterile sand for pupation for a period of eight days. Data collected included the total number of pupae recovered from each butternut, pupal weight based on individual weight of 100 pupa from each replicate, pupae per ml, percent adult emergence based on 30 adults from each replicate observed over a period of 14 days, fecundity and fertility, and flight ability. Each experiment was conducted for three generations.

Pupae were collected by sifting the sand daily for a period of three days. From the total number of pupae recovered, the weight of 100 pupae in grams from each species was measured on an analytical weighing machine and to further determine the size of the pupae, the volume of pupae per milliliter was measured using a volumetric cylinder. The pupae were transferred to petri dishes that were later placed in Perspex cages of 15cm by 15cm by 15cm dimension. Adults that emerged were given an adult diet which consist of sugar and enzymatic yeast hydrolysate in a 3:1 ratio and water on pumice granules adults were allowed seven days to enable full body coloration and maturation for mass rearing assessment. To determine fertility and fecundity, five pairs of adult fruit flies from the three species in a 1:1 female to male ratio age 7-10 days were placed in different Perspex cages of 15cm by 15cm by 15cm and exposed to an artificial plastic ovipositing device measuring 12cm height and 5cm diameter. The ovipositing device had tiny holes lined with paper towel saturated with butternut juice (80% concentration). The eggs were collected after 24 hours using a small painting brush and placed on a petri dish lined with a moist dark muslin cloth. Egg collection was done for 10 days and the number of eggs collected for each day was recorded, percent egg-hatch was observed over a period of three days under a microscope for three days. Flight ability was assessed based on 100 pupae from each replicate using the method of Taylor and Collin (2010) and Boller *et al*., (1981). The experiment was replicated four times for a period of three generations. Experiments were conducted in a room maintained at 27 ± 2°C, 60-70% Relative Humidity (RH) and photoperiod of 12 hours of light and 12 hours of darkness (L12:D12).

4. 4 Data Analysis

Data on host preference and mass rearing of *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* on butternut for three generations were first normalized and subjected to a two way analysis of variance (ANOVA) using R (Rauf *et al.*, 2013; Ekesi *et al.*, 2007). The means were later evaluated using Student-Newman-Keuls (SNK) test at α=0.05 probability levels.

4. 5 Results

4. 5.1 Host preference of tephritid fruit flies using choice test

Watermelon was equally preferred by all the three fruit fly species compared to the other host plants. For *B. cucurbitae* and *D. bivittatus* showed no significant difference in their preference of watermelon and butternut, while *D. ciliatus*, showed no significance difference in its preference of watermelon and courgettes (Table 4.1). Cucumber was the least preferred host plant by all the three fruit fly species as signified by the highest deformity percent and pupae/ml, lowest adult emergence percent and tibia measurement (Table 4.1). When the preference of the three fruit fly species was compared on butternut, watermelon, courgettes and cucumber, it was observed that

D. bivitattus, *B. cucurbitae* and *D. ciliatus* in that order perceived the host plants as suitable for their development (Table. 4.2)

Table 4.1. Means ±SE of number of fruit flies, their deformity percent, female percent and tibia measurements on various cucurbit crops in a choice test

Hostplants (Cucurbit)	Pupae recovered/Kg			Pupae/ml			Adult emergence (%)		
	B. cucurbitae	*D. bivitattus*	*D. ciliatus*	*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*	*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*
Watermelon	74.00±1.99^{Ab}	50.50±3.06^{Ac}	70.50±4.96^{Ac}	23.75±0.00^{Bc}	16.25±0.01^{Cb}	30.00±0.00^{Ab}	87.80±0.21^{Aa}	87.85±0.23^{Aa}	80.05±0.26^{Aab}
Cucumber	263.00±11.16^{Aab}	155.00±14.38^{Abc}	394±8.02^{Ab}	47.67±0.00^{Aa}	30.00±0.01^{Ba}	35.75±0.00^{Ba}	62.67±1.96^{Ab}	53.00±2.84^{Ab}	72.00±0.38^{Ab}
Courgettes	492.75±5.87^{Ba}	311.00±5.03^{Bab}	763.80±7.73^{Aa}	25.75±0.00^{Ab}	18.75±0.01^{Bb}	33.00±0.00^{Ab}	72.75±0.45^{Bab}	79.12±0.49^{ABa}	89.10±0.13^{Aa}
Butternut	504.25±10.41^{Aa}	462.00±4.12^{Aa}	139.25±7.55^{Bc}	28.50±0.00^{Bc}	21.75±0.00^{Cb}	30.00±0.00^{Aab}	86.88±0.11^{Aa}	87.45±0.11^{Aa}	73.60±0.52^{Ab}

Hostplants (Cucurbit)	Adult deformity (%)			Female (%)			Tibia length (mm)		
	B. cucurbitae	*D. bivitattus*	*D. ciliatus*	*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*	*B.cucurbitae*	*D. bivitattus*	*D. ciliatus*
Watermelon	4.35±0.08^{Ac}	3.88±0.11^{Ab}	4.73±0.07^{Aab}	55.47±0.18^{Aa}	54.52±0.18^{Aa}	55.25±0.28^{Aa}	0.70±0.00^{Ba}	0.78±0.00^{Aa}	0.58±0.01^{Aa}
Cucumber	24.67±1.11^{Aa}	28.20±1.81^{Aa}	8.78±0.30^{Aa}	50.57±0.47^{Aa}	42.95±2.59^{Aa}	55.70±0.28^{Aa}	0.49±0.00^{Bb}	0.54±0.00^{Ac}	0.51±0.00^{Bc}
Courgettes	11.65±0.35^{Ab}	12.18±0.48^{Ab}	2.28±0.11^{Bb}	48.68±0.25^{Aa}	53.75±0.36^{Aa}	53.45±0.15^{Aa}	0.54±0.00^{Bb}	0.65±0.00^{Ab}	0.53±0.00^{Bb}
Butternut	3.63±0.04^{ABc}	2.58±0.08^{Bb}	7.13±0.14^{Aab}	53.28±0.05^{Aa}	51.90±0.05^{Aa}	47.70±0.23^{Aa}	0.52±0.00^{Cc}	0.75±0.00^{Aa}	0.57±0.00^{Ba}

Means followed by the same lowercase letters within a column are not significantly different, SNK, α= 0.05. Means followed by the same uppercase letters within a row are not significantly different, SNK, α= 0.05.

Table 3.2 Total Mean ±SE of no. of fruit flies, their deformity percent, and female percent

Fruit fly species	Pupae recovered/Kg	Adult emergence (%)	Adult deformity (%)	Female (%)
B. cucurbitae	333.5±7.35[a]	77.53±0.00[a]	11.08±0.40[a]	50.78±0.24[a]
D. bivitattus	244.6±6.65[b]	76.86±0.00[a]	11.71±0.62[a]	50.78±0.80[a]
D. ciliatus	341.9±7.11[a]	78.6±90.00[a]	5.73±0.15[b]	53.02±0.24[a]

Means followed by the same letters within a column are not significantly different, SNK, α= 0.05

4. 5.2 Host preference of tephritid fruit flies using no-choice test

Watermelon was equally preferred by all the three fruit fly species compared to the other host plants. For *B. cucurbitae* and *D. bivittatus* showed no significant difference in their preference of watermelon and butternut, while *D. ciliatus*, showed no significance difference in its preference of watermelon and courgettes (Table 4.3). Cucumber was the least preferred host plant by all the three fruit fly species as signified by the highest deformity percent and pupae/ml, lowest adult emergence percent and tibia measurement (Table 4.3). When the preference of the three fruit fly species was compared on butternut, watermelon, courgettes and cucumber, it was observed that *D. bivitattus*, *B. cucurbitae* and *D. ciliatus* in that order perceived the host plants as suitable for their development (Table. 4.4)

Table 4.3. Means ±SE of the number of fruit flies, their deformity percent, female percent and tibia measurements on various cucurbit crops in a no-choice test

Hostplants (Cucurbit)	Pupae recovered/Kg			Pupae/ml			Adult emergence (%)		
	B. cucurbitae	*D. bivitattus*	*D. ciliatus*	*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*	*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*
Watermelon	143.50±1.99^{Ab}	73.50±1.71^{Bb}	113±2.92^{Ac}	24.25±0.00^{Bb}	17.75±0.01^{Cc}	30.50±0.00^{Aa}	80.18±0.25^{Aa}	84.65±0.82^{Aa}	89.05±0.14^{Aa}
Cucumber	340.00±15.01^{Aab}	303.50±20.12^{Aa}	332.50±7.82^{Ab}	43.00±0.00^{Aa}	33.00±0.01^{Aa}	35.75±0.00^{Aa}	53.93±0.43^{Ab}	63.00±0.72^{Aa}	74.60±0.29^{Aa}
Courgettes	624.75±11.89^{Aa}	814.75±10.51^{Aa}	715.25±11.17^{Aa}	27.00±0.00^{Ab}	20.75±0.01^{Aab}	33.25±0.00^{Aa}	71.47±0.34^{Ab}	64.40±0.68^{Aa}	84.20±0.29^{Aa}
Butternut	589.50±8.42^{Ba}	905.75±1.46^{Aa}	550.5±3.73^{Ba}	29.00±0.00^{Bb}	26.50±0.01^{Cbc}	32.00±0.00^{Aa}	79.47±0.22^{ABa}	88.60±0.10^{Aa}	73.78±0.23^{Ba}

Hostplants (Cucurbit)	Adult deformity (%)			Female (%)			Tibia length (mm)		
	B. cucurbitae	*D. bivitattus*	*D. ciliatus*	*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*	*B.cucurbitae*	*D. bivitattus*	*D. ciliatus*
Watermelon	0.90±0.03^{Ac}	2.28±0.15^{Aa}	0.80±0.04^{Ab}	53.18±0.04^{Aa}	54.50±0.25^{Aab}	52.78±0.09^{Aa}	0.69±0.00^{Ba}	0.78±0.00^{Aa}	0.56±0.00^{Ca}
Cucumber	19.37±0.23^{Aa}	10.35±0.21^{Aa}	7.63±0.29^{Aa}	45.57±0.82^{Aa}	59.75±0.14^{Aa}	49.62±0.33^{Aa}	0.49±0.00^{Ac}	0.53±0.01^{Ac}	0.51±0.00^{Ac}
Courgettes	7.00±0.20^{Ab}	5.20±0.24^{Aa}	1.28±0.05^{Ab}	47.75±0.25^{Aa}	48.88±0.17^{Ab}	55.95±0.35^{Aa}	0.53±0.00^{Ac}	0.64±0.01^{Ab}	0.53±0.00^{Ab}
Butternut	2.35±0.04^{Ac}	1.35±0.01^{Aa}	2.48±0.03^{Aab}	53.32±0.09^{Aa}	52.59±0.03^{Ab}	48.65±0.11^{ABa}	0.63±0.00^{Bb}	0.76±0.00^{Aa}	0.56±0.00^{Ca}

Means followed by the same lowercase letters within a column are not significantly different, SNK, α= 0.05. Means followed by the same uppercase letters within a row are not significantly different, SNK, α= 0.05.

Table 4.4 Total means ±SE of no. of fruit flies, their deformity and female percent

Fruitfly species	Pupae recovered/Kg	Adult emergence (%)	Adult deformity (%)	Female (%)
B. cucurbitae	424.43±9.32[b]	71.27±0.31[b]	7.41±0.13[a]	50.00±0.30[a]
D. bivitattus	524.38±8.45a	75.16±0.58[a]	4.80±0.15[b]	53.93±0.15[a]
D. ciliatus	427.81±6.41[b]	80.41±0.24[a]	3.05±0.10[c]	51.75±0.10[a]

Means followed by the same letters within a column are not significantly different, SNK, α= 0.05.

4. 5.3 Mass rearing of Tephrid fruit flies on butternut

The quality control parameters which were measured were pupae recovered/Kg of fruit, pupae weight, pupae/ml, percent adult emergence, fertility and fecundity, and flight ability. When these parameters were compared across the various fruit fly species that is *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* pupae recovered/Kg of fruit, adult emergence and fliers percents measured showed no significant difference (Table 4.5), while pupae/ml, pupae weight, fertility and fecundity showed significant difference (Table 4.5). Overall performance on butternut was greatest in *D. bivitattus* as the fruit fly species had significantly the highest pupal weight (g) and fecundity percent and the lowest pupae/ml followed by *B. cucurbitae* and *D. ciliatus* in that order (4.5).

Table 4.5. Means ±SE on the number of flies, fecundity percent, egg hatch percent and fliers percent of various fruit flies when reared on butternut

Parameters		*B. cucurbitae*	*D. bivitattus*	*D. ciliatus*
Pupae recovered/Kg fruits		334.35±1.214[a]	331.76±1.104[a]	283.81±1.065[ab]
Pupae weight (g)		0.015±0.045[b]	0.019±0.009[a]	0.013±0.076[c]
Pupae/ml		21.50±0.002[b]	19.75±0.003[c]	32.00±0.001[a]
Adult emergence (%)		94.17±0.327[a]	94.99±0.331[a]	88.06±0.420[b]
Fecundity (%)		70.81±0.185[ab]	78.17±0.208[a]	59.38±0.139[b]
Egg hatch (%)		90.29±0.135[a]	82.97±0.149[b]	81.2±0.184[b]
Fliers	10 cm tube (height)	86.91±0.096[a]	85.57±0.100[a]	88.23±0.102[a]
	15 cm tube (height)	82.2±0.100[a]	80.3±0.109[a]	82.4±0.081[a]

Means followed by the same lowercase letters within a column are not significantly different, SNK, α=0.05.

Pupal recovery of *B. cucurbitae, D. bivitattus* and *D. ciliatus* on butternut host plant significantly varied over the three generations observed. Pupal recovery for *D. bivitattus* was high for the first generation and significantly decreased in the second and third generation which were comparable (Table. 4.6) Pupal recovery for *D. ciliatus* increased over the three generations where the third generation had the highest pupae recovered (Table 4.6). Overall *B. cucurbitae* had the highest pupae (Table 4.6).

Percent adult emergence for *B. cucurbitae* and *D bivitattus* was significantly different for the generations and between the species, but was different from *D. ciliatus*. Percent adult emergence for *D. ciliatus* on butternut host plant marginally increased over the three generations with the third generation recording the highest percent (Table 4.6). Fecundity among the three tephritid fruit flies increased generally over the three generations (Table. 4.6). There was also an increase in egg hatch percent for the three tephritid fruit flies over the three generations (Table. 4.6)

There was significance difference on the flight ability of the three tephritid fruit fly species tested across the three generations when reared on butternut (Table. 4.7).

Table 4.6 Means ±SE on the number of fruit flies, fecundity percent and egg hatch percent when fruit flies are reared for three generations on butternut

Fruit fly Species	Pupae recovered Flies/Kg			Pupae weight (g)			Pupae/ml		
	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3
B. cucurbitae	381.9±1.911^{Aa}	298.3±2.60^{Aa}	322.8±1.547^{Aa}	0.014±0.081^{Bb}	0.016±0.048^{Ab}	0.016±0.062^{Ab}	21.5±0.004^{Ab}	23.5±0.005^{Ab}	22.8±0.005^{Ab}
D. bivitattus	404.8±1.490^{Aa}	293.8±1.209^{Ba}	296.7±1.237^{Ba}	0.018±0.017^{Ba}	0.019±0.046^{Aa}	0.019±0.040^{ABa}	19.75±0.006^{Ab}	19.50±0.05^{Ac}	21.0±0.006^{Ab}
D. ciliatus	257.9±1.321^{Bb}	241.7±1.260^{Ba}	351.9±0.994^{Aa}	0.011±0.140^{Bc}	0.013±0.048^{Ac}	0.012±0.066^{Ac}	32.00±0.003^{Aa}	30.7±0.003^{Aa}	31.6±0.003^{Aa}

Fruit fly Species	Adult emergence (%)			Fecundity			Egg hatch (%)		
	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3
B. cucurbitae	95.0±0.488^{Aa}	92.5±0.466^{Aa}	95.0±0.488^{Aa}	60.6±0.388^{Aa}	72.3±0.127^{Aa}	79.50±0.359^{Aa}	90.4±0.109^{Aa}	87.0±0.184^{Aa}	93.5±0.334^{Aa}
D. bivitattus	95.8±0.709^{Aa}	93.3±0.718^{Aa}	95.8±0.387^{Aa}	66.0±0.288^{Aa}	82.9±0.339^{Aa}	85.6±0.415^{Aa}	78.5±0.278^{Bb}	83.0±0.101^{Aa}	87.4±0.159^{Aa}
D. ciliatus	86.7±0.211^{Aa}	87.5±0.618^{Aa}	90.0±0.260^{Aa}	50.4±0.129^{Ba}	57.0±0.278^{Ba}	70.8±0.052^{Aa}	80.5±0.211^{Ab}	80.1±0.457^{Aa}	83.0±0.306^{Aa}

Means followed by the same lowercase letters within a column are not significantly different, SNK, α= 0.05. Means followed by the same uppercase letters within a row are not significantly different, SNK, α= 0.0

Table 4.7. Means ±SE on the flight ability of *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* across three generations when reared on butternut

Fruit fly Species	Fliers percent (10 cm height tubes)			Fliers percent (15 cm height tubes)		
	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3
B. cucurbitae	87.0±0.110^{Aa}	88.4±0.265^{Aa}	85.4±0.037^{Aa}	83.8±0.118^{Aa}	82.0±0.269^{Aa}	80.7±0.109^{Aa}
D. bivitattus	89.1±0.187^{Aa}	83.4±0.122^{Ab}	84.2±0.047^{Ab}	81.4±0.175^{Aa}	81.4±0.158^{Aa}	78.0±0.244^{Aa}
D. ciliatus	89.0±0.095^{Aa}	90.0±0.251^{Aa}	85.7±0.070^{Aa}	82.6±0.178^{Aa}	83.7±0.109^{Aa}	80.9±0.140^{Aa}

Means followed by the same lowercase letters within a column are not significantly different, SNK, α=0.05. Means followed by the same uppercase letters within a row are not significantly different, SNK, α=0.05

4.6 Discussion

4.6.1 Host preference of Tephritid fruit flies

Based on the number of adults that emerged from the pupae collected, *B. cucurbitae* and *D. bivitattus* showed strong preference for watermelon, butternut, courgettes and cucumber in that order. *D. ciliatus* preferred watermelon, courgettes, butternut and cucumber in decreasing order of preference. Tibia measurements similarly indicated that watermelon was the most suitable and preferred host for *B. cucurbitae, D. bivitattus*, and *D. ciliatus* in both the choice and the no choice test. Pupae size, adult emergence percent, deformity percent, sex ratio and tibia length have been used to determine host preference of tephritid fruit flies (Ren Li-Li1 *et al.*, 2008; Rauf *et al.,* 2013). Watermelon had also the least pupae/ml mean for all the three species, hence, was recorded as significantly preferred host. Body size of an insect is used as an indicator of its fitness and large insects are reported to be more competitive in mating, have a more dispersion capacity and high fertility (Navarro-Campos *et al.*, 2011; Thorne *et al.*, 2006).

Plant species differ in their suitability as food for insects. Given that the immature stages of developing insects are often rather immobile, the preference–performance hypothesis suggest that females should maximize the fitness of their offspring by laying their eggs on plant types on which the progeny perform the best (Jaenike, 1978; Thompson, 1988; Mayhew, 1997). Among tephritid fruit flies this host preference is guided by various parameters including odor, color, size and shape (Ren Li-Li1 *et al.*, 2008; Bruce *et al.*, 2005; Fletcher, 1987; Fitt, 1984; Hendrichs and Hendrichs, 1990).

Phytophagous insects have been observed to select oviposition sites based on the host suitability for the development of their offspring (Shikano *et al.*, 2010; Awmack and Leather, 2002). In this study, pupal recovery was high on cucumber than watermelon but it was not significantly different between butternut and courgettes for *B. cucurbitae*, *D. bivittaus* and *D. ciliatus*. In spite of this, cucumber had the least adult emergence percent, tibia measurements and the highest adult deformity for the three fruit fly species.

Adult emergence is dependent on the health of the pupae, while a pupa depends on the larvae. Poorly developed pupae mostly do not emerge to adults (Mayhew, 1997). A poor selection of host by adult increases deformity percent and reduces adult emergence percent. Several studies have also reported cases where female preference and performance appear uncoupled, or where the relationship is surprisingly weak (Fritz *et al.*, 2000; Faria and Fernandes, 2001). Several evolutionary and ecological considerations have been proposed to explain apparent mismatches between choice and performance including the fact that the strength of the preference–performance relationship is modified by ecological and ⁄ or life-history factors which may have contributed to the observations noted on cucumber in the current studies (Mayhew, 2001). All the tested fruit fly species are polyphagous; strictly attacking cucurbit. Within the context selecting for appropriate host, the female flies may encounter several constraints including limitations on the information processing capacity among similar host plant family (Cunningham, 2012). Nevertheless, insects like the species tested here should be better decision-makers than other phytophagous species attacking different plant families. However, it may take several generations for insects to adapt to certain host plants and the preference–performance link can be weak (or even negative) (Matsubayashi *et al.,* 2010), where insects are forced to or interact with plant species that they would ordinarily not prefer in nature and this may be the case observed for *B. cucurbitae* and *D. bivittaus* on cucumber.

4.6.2 Mass rearing of Tephrid fruit flies on butternut

Developing an efficient mass rearing procedure for *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* is vital in the research towards establishing a successful management program. The current studies showed that butternut fruit was among the preferred host plants of *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* and this host plant was assessed for whole fruit rearing of the three species of fruits flies. The study established that butternut is a suitable host for the mass rearing of the three fruit fly species. Host plants can be used in the mass rearing of tephritid fruit flies in the absence of an artificial diet (Ekesi and Mohamed, 2007). This is the first record of using butternut in the mass rearing of the three fruit fly species.

Based on the performance of the immature stages, butternut emerged as a suitable host for the mass rearing of the three fruit fly species. Mass rearing studies on other tephritid's fruit flies have used larval performance as a quality control parameter to show fitness of a diet (Ekesi and Mohamed, 2011; Hanife, 2008; Neto *et al.,* 2012; Ekesi *et al.,* 2007). Larval performance based on pupae recovered per kilogram of fruit, pupae per milliliter and pupal size were significant across all the three species. Immature stages i.e. larval performance have been used to illustrate the suitability of a diet or host plant for insects (Nash and Chapman, 2014). During host selection, female adults of phytophagous insects have been documented to select ovipositing sites based on the suitability of the host plant to support development and survival of its offspring (Sarwar *et al.,* 2013). However, larvae also have selective feeding depending on their preference (Fitt, 1984).

Percent adult emergence for *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* was greater than 70%. Butternut has been listed as among the preferred hosts of the three species in the wild (Badii *et al.,* 2015; Kumar *et al.,* 2006). Adult emergence in phytophagous insects is associated with the performance of the immature stages (Mayhew, 1997). During the development of artificial diets for mass rearing of tephritid fruit flies, adult emergence percent, fitness and health have been used to indicate the success of a diet (Ling *et al.,* 2006; Khan, 2013).

Fecundity and percent egg hatch for the three generations were observed to increase across the three generations for the three fruit fly species. Previous studies on *B. dorsalis* had associated the artificial rearing methods to prolonged laboratory adaptation of the different developmental stages (Ekesi *et al.,* 2007). In this case, the prolonged adaptation reflected in fecundity and egg hatch percent could be associated with the artificial methods of collecting and incubating the egg using extracted butternut juice. Fecundity has been interrelated with the larvae feeding that influences storage of resources that are important during the reproductive stage of the adult (Awmack and Leather, 2002). Hence, this indicated that butternut had the sufficient nutrients that are important in the reproduction stages of these insects. Egg hatch percent was >70% for the three species. Fertility of an insect is measured by the egg hatch success which is by the host plant or diet during the larval stage (Moreau *et al.,* 2006). Egg hatch percent is also key for the survival of each generation as it determines the population of the adults.

Measuring flight ability is important to ensure that insects reared in the laboratory are still viable and still possess qualities as wild insects. Flight ability of insects is important for dispersion and mating purposes. Percent fliers for *B. cucurbitae, D. bivitattus*, and *D. ciliatus* when reared on butternut was greater than 75%. During Sterile Insect Technique studies for the control of tephritid fruit fly species, flight ability has been used to compare performance of wild and domesticated fruit flies (Collins and Taylor, 2010). Other studies on tephritid fruit flies have used flight ability to determine the effectiveness of an artificial diet developed for mass rearing (Ekesi and Mohamed, 2011; Ekesi, *et al.,* 2007).

According to the Standards set by *icipe* (Ekesi and Mohamed, 2011) and FAO/IAEA/USDA(2003) on quality assurance parameters on regular weight of pupae of the same age using 100 pupae, adult emergence of greater than 70%, percent fliers of greater than 80%, percent egg hatch of greater than 80% show butternut as a suitable host for the mass rearing of the three fruit fly species.

4.7 Conclusion

The study indicated that although *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* are significant pests of cucurbit, they have host preferences when offered a variety of host plants. In this study, they significantly preferred watermelon over butternut, courgette, and cucumber. Therefore, selection of a host plant for insects is crucial for survival. In the absence of a preferred host, the three species were also observed to oviposit on the other host plants indicating the need for the management of the three species in all cucurbit.

Butternut was established as a suitable host plant for the mass rearing of the three fruit fly species in the absence of an artificial diet. This indicated that this host plant meets the standards set by Ekesi and Mohammed (2011) (*icipe*) and FAO/IAEA/USDA (2003) on quality assurance parameters for rearing tephritid fruit flies. It is listed as a host plant of these fruit fly species in the wild and is reported to be among the preferred hosts. Preference to butternut would be because of its richness with nutrients that are important in the growth and development of the immature stages and the reproduction stages of the adults. Butternut is a suitable rearing host plant due to its availability throughout the year with two seasons, they are cheap, are succulent enough to support the development of larvae and are of average size hence, very portable and can fit in any rearing cage with an opening of 15 cm diameter based on the previous host preference study which was conducted earlier in this chapter. This, therefore, shows the suitability of using butternut in the rearing of these tephritid fruit fly species.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATION

5.1 Discussion

The study on prevalence and diversity of tephritid fruit flies on cucurbit, indicated that *B. cucurbitae*, *D. ciliatus*, *D. bivitattus* and *D. vertebratus* are important pests of cucurbit. Previous on farm trap studies had associated these four species with yield losses on cucurbit farms. However, this is the first major report on fruit infestation (Ekesi and Billah 2007). *Bactrocera cucurbitae* had its highest infestation indices on bitter gourd and stripped melon. This fruit fly species was however dominant on the green melon, pumpkin and bitter gourd compared to the other three fruit fly species. Previous studies had listed bitter gourd (*M. charantia*), green melons (*C. melo*), and pumpkin (*C. maxima*) as among the preferred host plants of *B. cucurbitae* in the wild (Lanjar *et al.*, 2013; Amin *et al.*, 2011; Vayssières *et al.*, 2007). *Dacus ciliatus* had the highest infestation indices on cucumber (*C. sativus*) and pumpkin (*C. maxima*). However, courgettes (*C. pepo*), luffa (*L. cylindrical*) and pumpkin (*C. maxima*) were observed to better support the development of *D. ciliatus* compared to the other tephritid fruit flies. An earlier study by Vayssieres *et al.*, (2008) reported that *C. sativus*, *C. pepo* and *C. maxima* are the preferred and suitable hosts of *D. ciliatus* since they supported successful development of immature stages of this species (Vayssières *et al.*, 2008). The field study demonstrated that *D. bivitattus* preferred butternut (*C. moschata*) and bitter gourd (*M. charantia*) respectively. *Dacus bivitattus* was also observed to dominate the other species in these two crops. *Dacus bivitattus* is categorized as an economic major pest of cucurbit listing *M. charantia*, *C. moschata* and *L. siceraria* as among the preferred hosts of this fruit fly species in the wild (Badii *et al.*, 2015; de Meyer *et al.*, 2012). *Dacus vertebratus* had the highest infestation index on the stripped melon (*C. lanatus*) and a high relative abundance index on *C. lanatus* and the green melon (*C. melo*). In Africa, surveillance studies have shown that *C. melo*, *C. lanatus* and *C. maxima* farms succumb to significant yield losses due to *D. vertebratus* (Badii *et al.,* 2015).

During host preference studies, pupae size, adult emergence percent, deformity percent, sex ratio and tibia length were used to determine host preference of tephritid fruit flies (Ren Li-Li1 *et al.*, 2008; Rauf *et al.*, 2013). Based on the number of adults that emerged from pupae harvest, *B. cucurbitae* and *D. bivitattus* showed strong preference for watermelon, butternut, courgettes and cucumber in that order. *Dacus ciliatus*, however, preferred watermelon, courgettes, butternut and cucumber respectively. During the study tibia measurements similarly indicated that watermelon was the most suitable and preferred host for *B. cucurbitae*, *D. bivitattus*, and *D. ciliatus* in both choice and the no choice test. Watermelon also had the least pupae/ml mean for all the three species. Body size of an insect is used as an indicator of an insect's fitness; large insects are to be more competitive in mating, have a more dispersion capacity and high fertility (Navarro-Campos *et al.,* 2011; Thorne *et al.,* 2006). Plant species differ in their suitability as food for insects. Given that the immature stages of developing insects are often rather immobile, the preference–performance hypothesis suggests that females should maximize the fitness of their offspring by laying their eggs on plant types on which the progeny perform the best (Jaenike, 1978; Thompson, 1988; Mayhew, 1997). Among tephritid fruit flies, this host preference is guided by various parameters including odor, color, size and shape (Ren Li-Li1 *et al.,* 2008; Bruce *et al.*, 2005; Fletcher, 1987; Fitt, 1984; Hendrichs and Hendrichs, 1990).

Developing an efficient mass rearing procedure for *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* is vital in the research towards establishing a successful management program for these fruit fly species. Host plants have been used in the mass rearing of tephritid fruit flies in the absence of an artificial diet. During this study butternut was assessed for the whole fruit rearing of the three species of fruits flies and emerged as a suitable host for the mass rearing of the three fruit fly species. This is because pupae weight was regular, adult emergence was greater than 70%, egg-hatch percent was greater than 70% and percent fliers was greater than 80%, thus indicating that the insect colony was healthy as per the standards set by Ekesi and Mohammed (2011) (*icipe*) and FAO/IAEA/USDA (2003). In the wild, butternut is listed as a preferred host of *B. cucurbitae*, *D. bivitattus,* and *D. ciliatus* (Badii *et al.,* 2015; de Meyer *et al.,* 2012).

5.2 Conclusions

Bactrocera cucurbitae, *D. bivitattus*, *D. ciliatus* and *D. vertebratus* are significant insect pests of cucurbit and other horticultural crops in Coastal Kenya. Among the 10 cucurbit crops sampled in this study, bittergourd (*Momordica charantia*) and stripped melon (*C. lanatus*) are observed as the significantly preferred hosts of these three fruit fly species. These results indicate that there is a need for good strategic management method to improve yield production of these crops and prevent phytosanitary concerns that would ban these crops from being exported to other countries. When *Bactrocera cucurbitae*, *D. bivitattus*, and *D. ciliatus* were subjected to a free and no free feeding choice on butternut, courgettes, cucumber and watermelon, they were all observed to significantly prefer watermelon to the other host plants. Butternut emerged as a suitable host for the mass rearing of the three fruit fly species based on the standards set by *icipe* and FAO/IAEA/USDA (2003) on quality assurance parameters for rearing tephritid fruit flies.

5.3 Recommendation

Based on the findings of this study the following listed suggestion are recommended:

i. More research should be conducted on the diversity of *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* on other cucurbit farms in different regions of Kenya.

ii. Research on the yield losses caused by *B. cucurbitae*, *D. bivitattus* and *D. ciliatus* on cucurbit and other horticultural farms should be conducted.

iii. Host preference studies of *B. cucurbitae, D. bivitattus*, and *D. ciliatus* should also be conducted on other families to determine the host range of these fruit fly species in the absence of cucurbit.

iv. Further research on possible artificial diets for the mass rearing of *Dacus* species should also be conducted.

v. There is need for developing management strategies for tephritid fruit fly species to enable farmers reduce crop losses.

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