BIOECOLOGY AND MANAGEMENT OF FRUIT FLIES (*Dacus vertebratus* BEZZI AND *Zeugodacus cucurbitae* COQUILLETT) ON *Citrullus lanatus* (THUNB.) MATSUM. & NAKAI WITH ENTOMOPATHOGENIC FUNGI

BY

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ABSTRACT

Dacus vertebratus (Dv) and *Zeugodacus cucurbitae* (Zc) are important polyphagous Fruit Flies (FF) causing considerable yield loss in watermelon (*Citrullus lanatus*) worldwide. Entomopathogens have been used extensively for controlling insect pests due to environmental concerns to replace the use of chemical pesticides. However, information on the use of entomopathogens for FF control is scanty. Therefore, the life cycles of Dv and Zc, and their management with entomopathogens were investigated.

Life cycles of male and female Dv and Zc on watermelon were studied to determine Development Time-DT (days), morphometrics (mm) and survival (%) of immature stages following standard procedures. Eight commonly grown fruits and vegetables: watermelon, pawpaw, melon, cucumber, pickled-cucumber, caserta-zucchini, magda-zucchini and fordhook-zucchini were evaluated for susceptibility to Dv only due to non-susceptibility of Zc to other vegetable hosts apart from watermelon following standard methods. Fecundity and DT of Dv were assessed. Soil Samples (SS) were randomly collected from six main watermelon producing areas in Southern Benin Republic to identify and characterise local entomopathogens with capacity to control FF using standard procedures. Two commercial isolates of *Beauveria bassiana* (Bb337, Bb338) and *Metarhizium anisopliae* (Ma) were bio-assayed each at 10^5, 10^6, 10^7, 10^8, 10^9 and 10^{10} conidia/mL against Dv and Zc larvae for mortality. Probit analysis was computed to determine Lethal Concentration (LC_{50}). Efficacy of four isolated fungi from SS were evaluated at 10^{10} conidia/mL on both FF; mortality was assessed. Effectiveness of field application of Ma (50g/ha), Bb337 (50g/ha), Bb338 (50g/ha), ICIPE-69 (200mL/ha) and a synthetic insecticide (K-optimal, 10^3mL/ha) was evaluated on watermelon (25,000 plants/ha). Treatments were applied twice a week from three-weeks after sowing to two-weeks before harvest and yield (t/ha) data was collected. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Development of Dv and Zc was holometabolous with four stages. Male DT (16.6±0.3) and female DT (16.7±0.3) of Dv were not significantly different from Zc (male = 17.5±0.3; female = 18.8±0.4). *Dacus vertebratus* was smaller (female = 6.3±0.3 mm; male = 6.3±0.2 mm) than Zc (female = 6.4±0.3 mm; male = 8.2±0.2 mm). Survival rate of Dv and Zc were not significantly different for egg (Dv = 78.5%; Zc = 79.2%), first-instar (Dv = 72.7%; Zc = 71.7%), second-instar (Dv = 92.7%; Zc = 90.6%), third-

instar (Dv = 92.5%; Zc = 93.1%) and pupae (Dv = 85.5%; Zc = 80.7%). Eggs laid per mated-female of Dv were significantly higher on watermelon (650.5±50.2) followed by pawpaw (537.6±47.3), fordhook-zucchini (537.5 ± 46.3), cucumber (528.6±38.6), pickled-cucumber (528.1±39.2), melon (521.7±39.1), magda-zucchini (515.1±38.9) and caserta-zucchini (510.0±37.3). The DT of Dv was shortest on watermelon (16.6±1.3) < 16.7±1.6 (magda-zucchini) < 18.3±1.6 (caserta-zucchini) < 19.1±1.4 (fordhook-zucchini) < 19.6±1.9 (pawpaw) < 19.7±1.2 (cucumber) < 20.0±1.1 (melon and pickled-cucumber) suggesting host suitability. Four fungi species: *Aspergillus niger*, *A. flavus*, *Botryotricum* sp. and *Fusarium verticillioides* with entomopathogenic potential were identified from SS. Larval mortality was significantly higher with Ma (Dv = 37.5%; Zc = 63.1%), Bb337 (Dv = 31.8%; Zc = 39.2%) and least with Bb338 (Dv = 26.1%; Zc = 35.6%). The LC_{50} of larval stage of FF subjected to entomopathogens was 10^{10} conidia/mL. Highest mortalities were recorded on Zc (44.0%, 33.0%, 28.0%, 22.0%) treated with *A. niger*, *A. flavus*, *F. verticillioides* and *Botryotricum* sp. compared to mortalities on Dv (4.0%, 5.0%, 2.0%, 8.0%, respectively). Watermelon yield was superior with Ma application and was in the order: 148.8±25.2t/ha (Ma) > 145.8±25.2 t/ha (K-optimal) > 136.7±23.6 t/ha (Bb337) > 119.2±20.5 t/ha (Bb338) > 115.8±20.9 t/ha (ICIPE69).

Watermelon was the most susceptible host fruit of *Dacus vertebratus* and *Zeugodacus cucurbitae. Metarhizium anisopliae* at 50g/ha was sufficient to control the insect, thus increasing watermelon production above conventional pesticide.

Keywords: Fruit fly, *Citrullus lanatus*, *Metarhizium anisopliae*, *Beauveria bassiana, Aspergillus niger*.

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CERTIFICATION

I certify that this research work was carried out by Micheline Vignon **HINTENOU** under my supervision in the Entomology unit of the Department of Crop Protection and Environmental Biology, Faculty of Agriculture, University of Ibadan; Agricultural Entomology Laboratory of the University of Abomey-Calavi, International Institute of Tropical Agriculture (IITA) and the Directorate of Vegetable Production in Benin Republic.

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DEDICATION

This work is dedicated to my husband, A. M. T. DANGBENON, daughters Maëlys H. Oladikpikpo A. and Ornella S. Iréwolé Iris for their love, everlasting sacrifices and unlimited supports. May you stay blessed.

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CHAPTER ONE

INTRODUCTION

Agriculture remains a subsistence activity for many people worldwide. It plays an important role in human welfare in a given country with approximately 78% (about 1.3 billion) of the world's active population directly depending on it as source of income to support family needs (World Bank, 2016). The sector contributes to economic growth as a supplier of raw materials for industry, indigenous industrial products and is also a substantial source of foreign export earnings (Raisbeck, 2003, Klasen *et al.,* 2016). Agriculture represents a major part of the economies of all African countries and can help in addressing major regional priorities, such as poverty and hunger eradication, sustainable management of resources and environment, and job creation. In Benin, agriculture is the largest economic sector where it accounts for 22.64% of the Gross Domestic Products (GDP) (World Bank, 2019) and generates 70% of employment (IFAD, 2019). It contributes predominantly to the national income for most developing countries through the availability of fodder for domestic animals and agricultural products for human consumption (Khanna and Solanki, 2014).

Among the large array of agricultural commodities, fruits and vegetables production are among the largest sectors of the industry of horticulture in Africa (Mergenthaler *et al.,* 2009; AGRA, 2016). Over 100 million tonnes of tropical fruits were produced worldwide in 2018 (Altendorf, 2019). Fruits and vegetables have nutritional values as they are rich in vitamins, minerals, fibre and other bio-active compounds which provide energy (Amao, 2018). Different types of fruits and vegetables crops including pineapple, mango, avocado, citrus, pawpaw, banana, garden eggs, tomatoes, okra, pepper and cucurbits are grown in Africa for domestic needs as well as export markets (Kadio *et al.,* 2011). Most commonly cucurbits grown worldwide include watermelon (*Citrullus lanatus* Thunb.), zucchini (*Cucurbita pepo* L.), cucumber (*Cucumis sativus* L.), honey melon (*Cucumis melo* L.), bitter gourd (*Momordica charantia* L.) and so on.

(Renner and Schaefer, 2016). They are consumed in various forms and are rich in different vitamins (A, C, E), zinc, iron, carbohydrates, proteins, lipids, minerals, fiber, and antioxidant (Afari-Sefa *et al.,* 2016; Howélé *et al.,* 2018). Cucurbitaceae family constitute the largest group of tropical vegetables and ranks highest among the plant families with regard to number and percentage of species used by humans (Schippers, 2004). Watermelon represents the most cultivated crops worldwide with the total consumption greater than that of any other cucurbits (FAO, 2018).

Fruits and vegetables despite their importance, are among the most important agricultural commodities that are attacked by insect pests reducing in significant reduction in productivity and availability (Boisclair and Estevez, 2006; Phophi and Mafongoya*,* 2017). Tephtritidae fruit flies represent the most damaging pests especially because of their diversity, high reproductive rate and polyphagous nature (Ekesi *et al.,* 2011). Species belonging to tribe Dacini represent serious pests causing significant horticultural crops losses in terms of quantity and quality worldwide (Dohino *et al.,* 2016). Three economically important genera include *Bactrocera*, *Dacus* and *Zeugodacus* in which about 932 species have been described (Doorenweerd *et al.,* 2018). Economically, direct damage ranged between 30 and 100% which considerably vary with the maturity and variety of the fruit (Vayssières *et al.,* 2013; Vargas *et al.,* 2015). Fruit flies cause important economic losses to different crops such as: *Psidium guajava* L. (Myrtaceae), *Mangifera indica* L. (Anacardiaceae), *Citrus* spp. (Rutaceae), *Cucurbita pepo* (Cucurbitacae), *Cucumis sativus* (Cucurbitacae) and *Citrullus lanatus* (Cucurbitacae) (Ekesi *et al.,* 2016). On cucurbit plants such as *C. pepo* and *Solanum lycopersicum,* indigenous species induced up to 53% yield loss (Kambura *et al.,* 2018). The most damaging species being reported on Cucurbitaceae plants include *D. punctatifrons* Karsch, *D. vertebratus* Bezzi, *Dacus ciliatus* Loew, *D. bivittatus* Bigot, *Zeugodacus cucurbitae* Coquillett (Gnanvossou *et al.,* 2005; Freidberg *et al.,* 2017). *Dacus vertebratus* and *Z. cucurbitae* are the most damaging vegetable pest of cucurbits crops especially on watermelon, *C. lanatus* (Gnanvossou *et al.,* 2005; Biokou *et al.,* 2015; Sarwar, 2015). In addition, indirect losses are associated to fruit flies infestation with quarantine restrictions inflicted by importing countries of fruits and vegetables (APHIS-USDA, 2009; Vasudha and Agarwal, 2019).

Fruit flies management has seen the development and proliferation of techniques, methods and strategies for the suppression of their population. On this basis, an integrated pest management (IPM) strategy has been developed and involved at least two control measures to reduce the fruit flies' population to tolerable level (Ekesi *et al.,* 2016). These control measures include chemical control, monitoring with attractants, cultural control (field sanitation, fruit bagging, prompt fruit harvest, post-harvest treatment), soil inoculation, release of natural enemies and sterile insects (Badii *et al.,* 2015; Vargas *et al.,* 2015). As a component of IPM programme, the use of chemical attractants (trimedlure, methyl eugenol, cue-lure) and food attractants (Torula yeast, CeraTrap) have shown their specificity in capturing fly species (Bárbara *et al.,* 2011; Minhibo *et al*., 2018). Unfortunately, chemical control is not effective in stopping the damage caused by the flies because of their complex biology and the adverse effects of insecticide application on the environment, producers and consumers (Pierre *et al.,* 2015; Minhibo *et al*., 2018). In addition, the problem of insecticide resistance has become a major constraint in controlling insect pest (Jallow *et al.,* 2017). Reflections have then been made towards more friendly strategy to gather additional information to tolerate the use of insecticides and to supplement with other methods known to be safer to reduce fruit flies' population (Dimbi *et al.,* 2009). Biologically based forms of pest control through the utilisation of entomopathogenic fungi were used as substitute for an extensive management of fruit flies (Flores *et al.,* 2013; Toledo *et al.,* 2017).

Fungal agents are the most propitious group among biocontrol agents. They have been used worldwide to reduce population of many agricultural pests and some have been developed and targeted against fruit flies (Elbashir *et al.,* 2014; Hadi *et al.,* 2017). The use of entomopathogenic fungi in Tephritid fruit fly management includes aerial applications targeting adults and also soil treatment targeting third instar larvae and pupae (Hallouti *et al.,* 2017). The effectiveness of these agents according to a modern approach, should be applied in localized spots and their dissemination would rely on the easy dispersion of conidia from infected to uninfected hosts (Imoulan *et al.,* 2016). However, the effectiveness of a microorganism for the successful suppression of an insect pest depends on its efficacy in suppressing the pest and on low or no effect on non-target organisms. *Beauveria bassiana* (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff) are known as one of the most important entomopathogens

of dipteran insects (De La Rosa *et al.,* 2000; Maina *et al.,* 2018). The current work focuses therefore on prospecting for native microflora with potential as entomopathogens from soil sample in cucurbit orchards and assessment of existing isolates for the control of the fruit flies species infesting *Citrullus lanatus*.

The present work aims to investigate potential entomopathogenic fungi in the local community and to evaluate their efficacy against fruit flies for the development of better strategies in integrated fly fly management. The objectives of this work are to:

1. Assess the occurrence, abundance and diversity of entomopathogenic fungi associated with soils used for growing vegetables in Benin Republic
2. Investigate the development and reproductive biology of *Dacus vertebratus* and *Zeugodacus cucurbitae* on *Citrullus lanatus*
3. Study the aspects of the ecology of *Dacus vertebratus* and *Zeugodacus cucurbitae*
4. Evaluate the efficacy of fungi with entomopathogenic potential for the control of *Dacus vertebratus* and *Zeugodacus cucurbitae*

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CHAPTER TWO

LITERATURE REVIEW

2.1 Agronomic Characteristics of Cucurbits Crops

The term "Cucurbit" is generally used to characterise species belonging to the family of Cucurbitaceae. About 800 species within 130 genera are described in this family (Shah *et al.,* 2014). The Cucurbitaceae are cultivated all over the world for their fruits which are known as the most consumed fruits worldwide and particularly in Africa (Andolfo *et al.,* 2017; Salehi *et al.,* 2019). Cucurbit fruits are rich in several vitamins and minerals (Wang *et al.,* 2011). All cucurbits, except the bottle gourd, have bright yellow flowers and each vine produces a male and a female flower. Most commonly cucurbit crops include cucumber, melon, watermelon, summer squash, pumpkin, zucchini and sponge gourds, calabash, bitter melon, luffa, cantaloupe, cashew, and many others (McCreight *et al*., 2016).

2.2 *Citrullus lanatus* Thunb. (Cucurbitales: Cucurbitacae)

Citrullus lanatus is a climbing plant (scrambler) and a domesticated watermelon (Laghetti and Hammer, 2007; Paris, 2017). It is botanically considered as a fruit which belongs to the family Cucurbitaceae (Edwards *et al.,* 2003). Cucurbitaceae family ranks very high among the most important plant worldwide and a number of species are used in human nutrition. The global consumption of watermelon is higher than any other crops pertaining to cucurbit (FAO, 2017). It is so called as watermelon because of large amount of water it contains, which is about 93% of weight (Erhirhie and Ekene, 2013). Over 1,200 varieties of watermelon worldwide and quite many of these varieties are also cultivated in Africa (Ogwu *et al.,* 2016).

2.2.1 Classification and botanical description of *Citrullus lanatus*

Citrullus lanatus appertains to the kingdom, Plantae; division: Magnoliophyta; Class: Magnoliopsida, Order: Cucurbitales and Family: Cucurbitaceae (Mercy and Bosa, 2013). It is an annual plant having their limbs spread out with coarse. The leaves are large, hairy with pinnately lobes (Wehner *et al.,* 2001). The seeds are small measuring

in length (0.4 - 0.11 cm) and in width (0.2 - 0.3 cm). They are lightly brown-white in colour, smooth, hairy and of about 7 - 15 cm in diameter (Jain *et al.,* 2013). The fruits are roughly spherical in shape with shallow grooves having a greenish yellow skin and measured 14 - 20 cm in length (Wehner *et al.,* 2001).

2.2.2 Origin and distribution of *Citrullus lanatus*

The origin of watermelon is known to be Kalahari in Namibia and the deserts of Sahara in Africa (Jarret *et al.,* 1996). Nowadays, watermelon is produced abundantly in world tropical regions (Renner, 2015). More than 96 countries grow watermelon worldwide. China ranked first producers all over the world having 70.3% of the global production in 2017 followed by Turkey (4.70%), Iran (4.30%), the United States (3.20%) and Egypt (1.80%) (FAOSTAT, 2017).

2.2.3 Economic importance and nutritional value of *Citrullus lanatus*

In West Africa, Nigeria produced more watermelons in 2011 (139,223 tons) as compared to Kenya, which produced 66,196 tons and South Africa that produced 77,993 tons (This Day Live, 2014). The fresh watermelon is low in calories, highly nutritious, sweet containing 93% water and 8% sugar (Enujeke, 2015). It contains Vitamins (C and A), Minerals, Proteins, Potassium, Lycopene and is fat free (Medicine Net, 2004). Apart from nutrient value, watermelon is also important as natural medicine source (Ignjatovic, 2005). The flowers of watermelon provide an excellent source of nectar and pollen for bees (McGregor and Waters, 2014).

2.2.4 Cultivars of *Citrullus lanatus*

In tropical Africa, several cultivars of watermelon are available. In Benin, among the most important four majors' cultivars: watermelon var. Kaolack, Sugar-baby, Logone and Charleston gray are produced.

2.2.4.1 *Citrullus lanatus*, cultivar Kaolack

The fruit of this cultivar is round and weighted about 5 to 6 kg. The epidermis is lightly green in colour having fine medium stripes (Plate 2.1a). It has red flesh colour which is crunchy, smooth, sweet and well-appreciated by consumers. With a high yield and a good behaviour under different conditions, Kaolack is famous in Sub-Saharan countries. The maturity days are 80-85 days from transplant to harvest (Aniekwe and Nwokwu, 2013).

2.2.4.2 *Citrullus lanatus*, cultivar Sugar baby

The fruits of this cultivar are 15 to 20 cm in diameter and weighted about 8 kg. The epidermis is very thick and dark green which becomes almost black when ripe (Plate 2.1b). The flesh is sweet, red, firm, and crisp with very few and small seeds. The seeds of this cultivar are dark brown in colour. It has a good productivity and precocity. This cultivar is native to the USA and was introduced in 1956 in Oklahoma. Sugar baby is an early maturing variety, maturing in 75-80 days. Sugar Baby watermelons rank as one of the sweetest watermelon cultivars (Grant, 2018).

2.2.4.3 *Citrullus lanatus*, cultivar Charleston gray

The fruit of this cultivar is oblong to cylindrical weighting 6 to 15 kg. The epidermis is gray to green and slightly veined (Plate 2.1c). The flesh of the fruit is pink to red in colour while the seeds are brown. It is grown in 75 to 80 days. It is originated from USA and introduced in 1963 in Southern Carolina from the breeding of "Sugar Baby" and "Charleston Gray" (www. planfor.fr/achat pastèque).

2.2.4.4 *Citrullus lanatus*, cultivar Logone

The fruit of this cultivar is obtained from the new F1 cultivar that combine better agronomic traits with greater disease resistance. It is a result of a hybrid from the crossing of 'Sugar Dragon' and 'Sunshine' (seedless) and being introduced to African market. The fruits of this cultivar weight about 9 to 10 kg. They are oval with red flesh (Plate 2.1d) (www. planfor.fr/achat pastèque).

2.3 Tephritid Fruit Fly Infesting Watermelon, *Citrullus lanatus*

Fruit flies (Dipteran: Tephritidae) consist of over 4000 species worldwide and are known to cause economic losses through fruit infestation, quarantine restrictions and regulations (Vargas *et al.,* 2010; Benelli, 2015). In Africa, especially Sub-Saharan Africa (SSA), about 915 species belonging to 148 genera are recorded (Ekesi *et al.,* 2016). Major fruit fly pests which attack commercial fruit and vegetable are found in both *Ceratitis* and *Dacus* genera (Badii *et al.,* 2015). Considerable losses on cucurbits are caused by *Dacus* species such as: *D. vertebratus* (Bezzi), *D. bivittatus* (Bogot), *D. lounsburyii* (Coquillet), *D. ciliatus* (Loew). (De Meyer *et al.,* 2012; Inskeep *et al.,* 2018). The intensive increase of fruits exchange across borders, African continent has become most susceptible and defenceless to the entry of non-native fruit flies species.

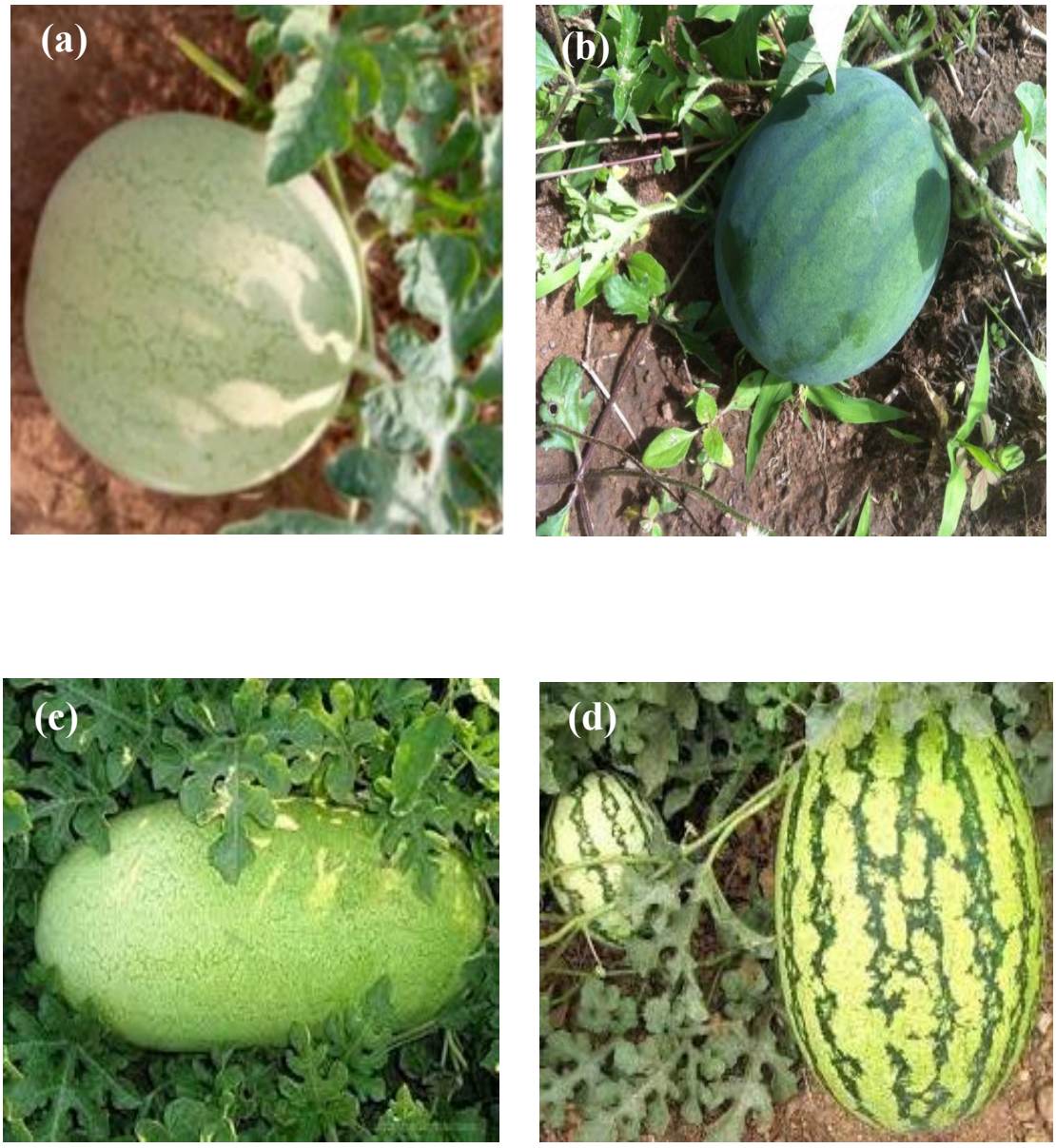


Plate 2.1. Cultivars of *Citrullus lanatus*

Source: Biokou *et al.* (2015)

a = watermelon var. Kaolack

b = watermelon var. Sugar baby

c = watermelon var. Charleston gray

d = watermelon var. Logone

2.3.1 Life cycle of Tephritid Fruit Flies

All fruit fly species undergo four instars development including eggs, larvae, pupae and adults during their life cycle in four to five weeks. Eggs laid by the female are in batch of 1 to 20 which are deposited under the skin of a ripening fruit (Weems, 2002). Within 2 to 4 days, eggs hatching occurs and the first instar larva feed on the flesh of fruit (Amur *et al.,* 2017). This induces a premature ripeness and rotteness of the infested fruit. The larval stage represents the most likely instar to recognize the presence of fruit fly in the fruit when open (Duyck, 2005). As the fruit ripens and rots, fully mature larvae jump into the soil to change into pupae stage from which adult flies emerged within 7 to 15 days (HORTGRO, 2016; Amur *et al.,* 2017). The duration of the life cycle depends on temperature whereby cool temperatures slow the development while high temperatures speed up development (Niassy *et al.,* 2012).

2.3.2 Characteristics of the Tephritid Fruit Fly damage

Tephritids fruit fly are polyphagous, causing serious injuries to fruit and vegetables (Ben-Yosef, 2015) (Plate 2.2). Direct injuries are caused during oviposition where flies introduced into the fruit a gut bacterium which causes rotting of the tissues that surround the eggs (Capuzzo *et al.,* 2005; Kounatidis *et al.,* 2009; Naaz *et al.,* 2016; Yong *et al.,* 2017a, b). This results in the reduction of the quality and marketable value of the fruits. Also, the feeding activities of the larvae lead to a soft and pasty mess. The punctures favour the infection by pathogens resulting in a rapid development and increase of the fruit's decay (Ventura *et al.,* 2018; Zhao *et al.,* 2018). The second or third instar larvae inflict quantitative damage to fruits by consuming the pulp resulting in yield and quality losses at harvest (Ekesi *et al.,* 2016). The presence of a single maggot inside fruits assigned for export often lead to quarantine restrictions and regulations in order to keep fruit flies from entry into a country (Sarwar *et al.,* 2015). Heavy losses are encountered in fruit production in Africa due to infestation by fruit fly (Grove and Beer, 2014; Qin *et al.,* 2015). Considerable economic damage are mainly inflicted by invasive alien species inducing non-expected side effects on endogenous fruit flies populations (Lyon and Miller, 2000; Oliveira *et al.,* 2013).

2.3.3 Economic importance of Fruits Flies

More than 900 fruit fly species of 148 genera, with about 300 species infesting not domesticated and / or grown hosts (Ekesi *et al.,* 2016). They constitute an important

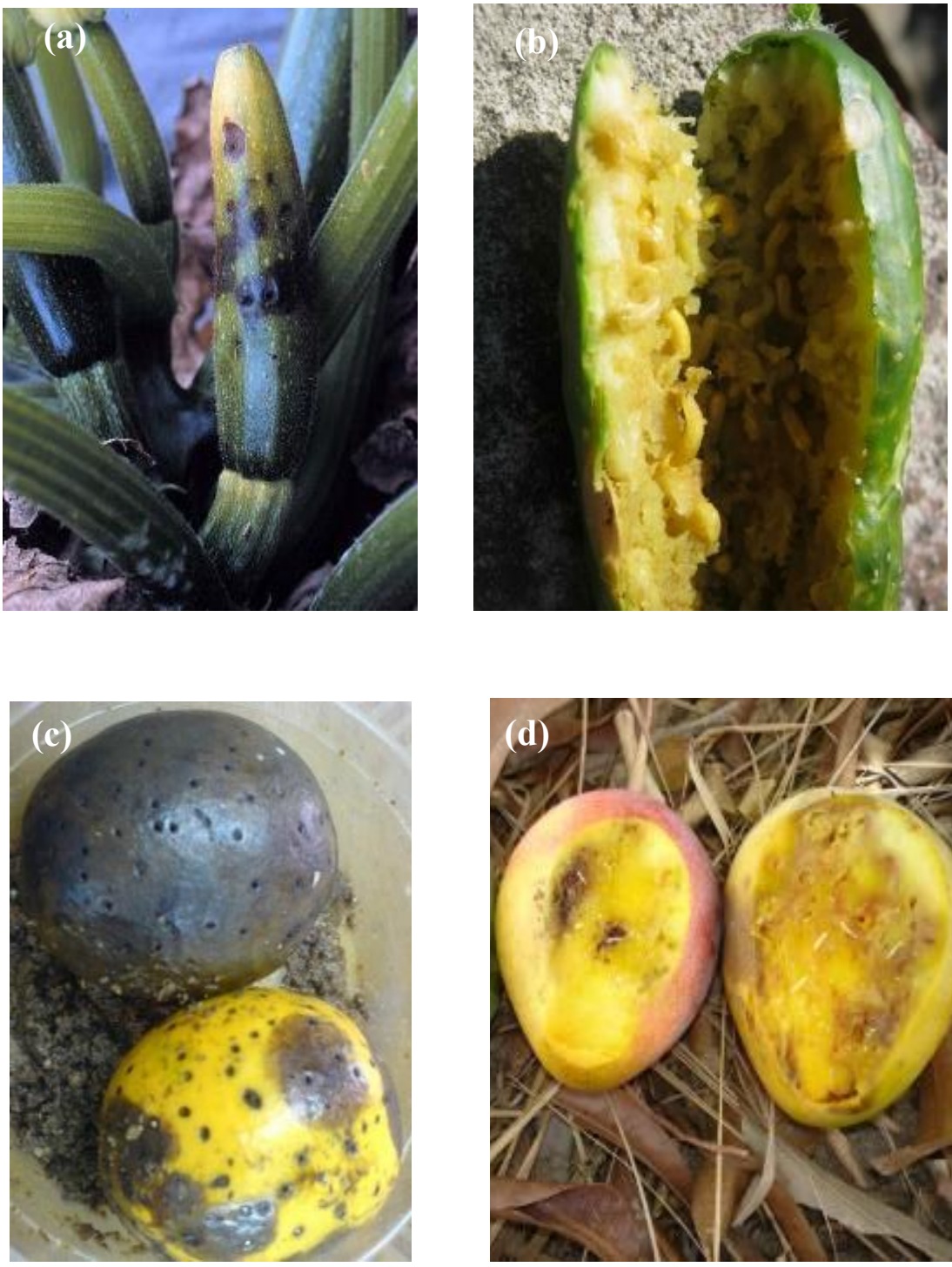


Plate 2.2. Damages caused by Tephritids flies

a = Fruit fly damages on *Cucurbita pepo* (Ekesi and Billah, 2006)

b = Cucumber damage due to cucumber fruit fly larvae (Ellis *et al.,* 1996)

c = *Irvingia gabonensis* infested by *Bactrocera dorsalis* (Hintènou, 2011)

d = *Mangifera indica* infested by *Bactrocera dorsalis* (Vayssières *et al.,* 2008)

constraints to horticultural production causing unimaginable crops losses in most Africa countries (Muhammad and Kiilu, 2004; Carey *et al.,* 2017). Fruit flies interfere with fruits and vegetables production, availability and marketability by reducing their quantity and quality. They are easily moved across borders because of lack of a careful search (ICIPE, 2007). This gives them the unique status of a quarantine pest worldwide. The new regulations from European countries importing fruits and vegetables impose strict quarantines measures which have as a consequence the rejection of mango consignment with the presence of a single larva. This may lead to a possible prohibition for the exporting country. Annual economic losses of up to 42 million USD has been estimated in Africa and about 1 billion USD worldwide (Badii *et al.,* 2015; Dohino *et al.,* 2016). Mangoes exportation have been forbidden from several countries of Sub-Saharan Africa to Europeans and United States of America markets (Manrakhan, 2016). In Africa, several native species are of economic importance and caused severe damages to crops such as : *Zeugodacus cucurbitae* Coquillett, *Dacus bivittatus* Bigot, *Dacus vertebratus, Ceratitis capitata* Wiedemann, *Ceratitis rosa, Ceratitis cosyra* Walker, *Dacus ciliatus* Loew ((Deguine *et al.,* 2015; Kambura *et al.,* 2018). Important native species such as *D. vertebratus* and *Z. cucurbitae* are especially important on watermelon causing 70 – 100% fruits losses (Mwatawala *et al.,* 2015).

2.4 Jointed Pumpkin Fly, *Dacus vertebratus* Bezzi (Diptera: Tephritidae)

2.4.1 Classification and morphological description of *Dacus vertebratus*

Dacus vertebratus belongs to the Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Diptera, and Family: Tephritidae (Norrbom *et al.,* 1999). *Dacus vertebratus* is orange to brown in colour. The medium size of the species is about 7-9 mm in length for 12-15 mm wingspan. The width of male and female's head are the same (Plate 2.3). *Dacus vertebratus* has an elongated eye. It has its scape, pedicel, first flagellomere scape and pedicel short while the first flagellomere is elongated (de Meyer *et al.,* 2012). On the thorax, the scutum is red to brown in colour without yellow strip and a large dark central stripe which broadens basally. The posterior-lateral of the thorax has two orange strips below the scutellum which is yellow and densely setulose. The abdomen of *D. vertebratus* is parallel sided with abdominal tergites fused (Caroll *et al.,* 2002).

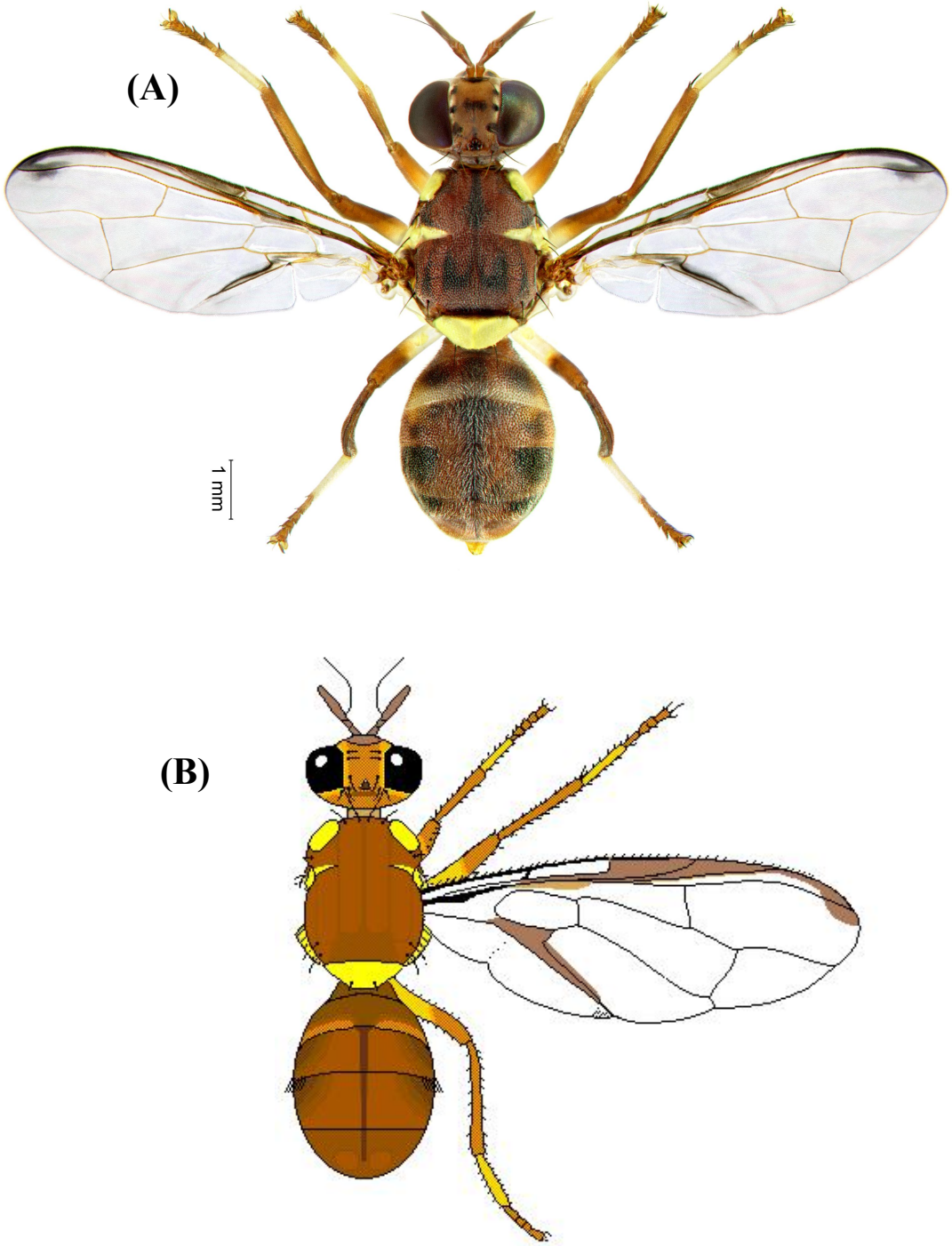


Plate 2.3. *Dacus vertebratus* Bezzi (Diptera: Tephritidae)

(A) Female of *D. vertebratus* (Photo Credit by Goergen, IITA)

(B) Male of *D. vertebratus* (Caroll *et al.,* 2002)

2.4.2 Origin and distribution of *Dacus vertebratus*

*Dacus vertebratu*s is known to have originated from Africa (de Meyer *et al.,* 2012). It occurs in Afro-tropical countries, Madagascar, the Arabian Peninsula. Mayotte (White, 2006). It is well distributed in Africa, Indian Ocean island and Middle-East as well as in several others countries including Angola, Benin Republic, Cameroon, Ethiopia, Gambia, Ghana, Kenya, Liberia, Malawi, Nigeria, Senegal, Southern Africa, Tanzania, Zambia, Zimbabwe, Saudi Arabia, Yemen, etc. (White and Elson-Harris, 1992).

2.4.3 Host range and damage caused by *Dacus vertebratus*

Injury caused by *Dacus vertebratus* to cucurbits plants is comprised between 25% and 100% depending on the crops (Badii *et al.,* 2015). In Africa, most of significant yield losses observed in watermelon and pumpkin farms are due to *D. vertebratus* (Badii *et al.,* 2015). The most preferred host of *D. vertebratus* is watermelon. Female adults lay eggs inside the flesh of the very young fruit of watermelon (about 2 cm) resulting in the huge yield losses which may be estimated in billions of dollars. Severe infestation occurs often after the formation of young fruits (Norrbom, 2004; Biokou *et al.,* 2015).

2.5 Melon Fly, *Zeugodacus cucurbitae* Coquillett (Diptera: Tephritidae)

2.5.1 Classification and morphological description of *Zeugodacus cucurbitae*

Zeugodacus cucurbitae was formally described as *Dacus cucurbitae* by Coquillett in 1899 It was later placed in the sub-genus *Zeugodacus* according to previous authors (Drew, 1973). Later on, it was classified under *Bactrocera* genus (Drew, 1989). Its systematic position was recently reviewed as *Bactrocera* (Krosch *et al.,* 2012). It has been recently placed in the genus of *Zeugodacus* by Virgilio *et al.* (2015).It is placed into the Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta Order: Diptera; Suborder: Brachycera; Division: Cyclorrapha; Family: Tephritidae; Sub-family: Dacinae and Genus: *Zeugodacus* (CABI, 2017, Doorenweerd *et al.,* 2019). Adult of *Z. cucurbitae* is predominantly orange-brown. The medium length and wingspan of the species is about 8.0 - 10.0 mm and 14.0 - 16.9 mm respectively. The male is small in size as compared to the female. The head is characterized by an antenna which is longer than the face. The face is yellow with moderate rounds spots which are dark in colour and situated in each antennal furrow. The scutum on the thorax is mainly a mixture of red and brown. The medial and lateral post-sutural vitta are yellow. The

scutellum is completely yellow except the basal margin which is dark (Plate 2.4). All tergites of the abdomen is separated (tergites I–V) with a medial T-shaped mark present. On the legs, coxae are brownish with a bicoloured and pale femora and basally yellow (Caroll *et al.,* 2002; Drew and Romig, 2013).

2.5.2 Origin and distribution of *Zeugodacus cucurbitae*

Zeugodacus cucurbitae is originated from Asia. It has been found in tropics and sub-tropics sides of Africa. Its presence has been prompted in some areas in Indian Ocean, Australia and Pacific Islands and Hawaii (EPPO, 2018). It is present in some Regions of Australian and Oceanian (Drew and Romig, 2013). It has established in Seychelles (White *et al.,* 2001). In Africa, it was primary registered in Tanzania (1936) and Kenya (1937) (Bianchi and Krauss, 1937; Gilbert, 2004). It is now found in most African countries including Tanzania (1936), Kenya (1937), Ethiopia (2010) and Sudan (2010) (Vayssières *et al.,* 2007). It has been in Malawi (2010) and northern Mozambique in 2013. In west Africa, it has been found in Côte d'Ivoire (1999), Mali (2000), Burkina-Faso (2000), Nigeria (2001) and Benin Republic (2004) (de Meyer *et al.,* 2015).

2.5.3 Host range and damages caused by *Zeugodacus cucurbitae*

Zeugodacus cucurbitae is a key pest of Cucurbitaceae including cucumber, melon, watermelon and bitter gourd. About 45 species of nine families are considered and recognised as hosts in Africa and in the Indian Ocean (Vayssières *et al.,* 2007). Twenty-nine of them are *Cucurbitaceae* particularly cucumber was the most preferred host in West and East Africa (Mwatawala *et al.,* 2010). *Zeugodacus cucurbitae* was collected from non-cucurbits host plants namely: *Mangifera indica* (Anacardiaceae), *Prunus persica* (Rosaceae), *Citrus sinensis* (Rutaceae), *Averrhoa carambola* (Oxalidaceae), *Capsicum annuum* (Solanaceae), *C. frutescens* (Solanaceae), *Solanum lycopersicum* (Solanaceae), etc. (de Meyer *et al.,* 2015).

2.6 Management of fruit fly infestation

Several methods have been developed and used to control tephritid fruit flies all over the world. Several control actions include cover sprays, protein bait sprays, male annihilation; sterile insect releases; releases of natural enemies etc. The commonest are orchard sanitation, chemical control, cultural control, and Biological control.

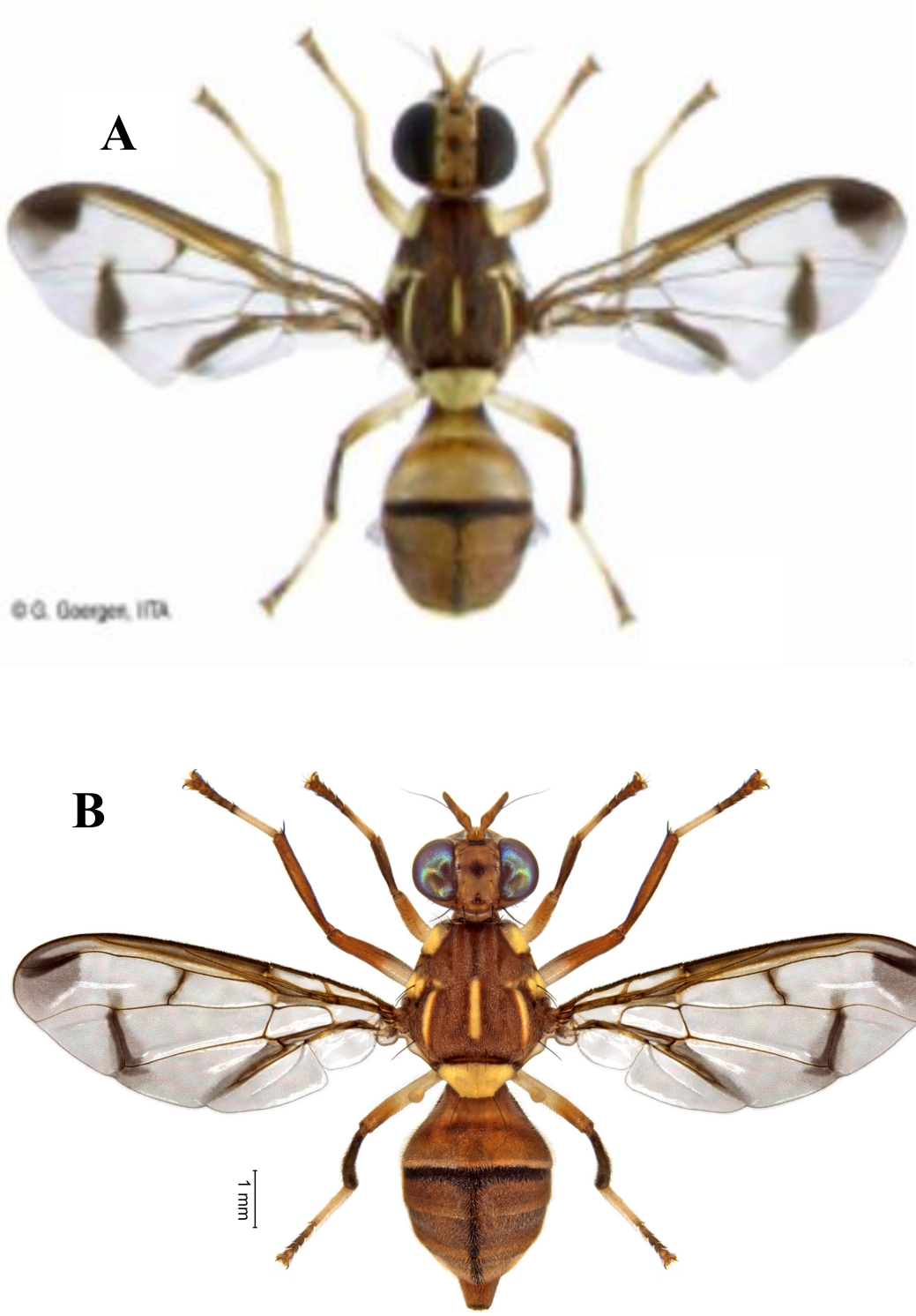


Plate 2.4. *Zeugodacus cucurbitae* (Diptera: Tephritidae) A) Male and B) Female

Source: Photos credited by Goergen, IITA

2.6.1 Chemical control of Fruit Flies

Synthetic pesticides for controlling fruits flies is a commonly practice used to protect horticultural crops in fields (Ekesi *et al.,* 2007). Despite the disadvantages of pesticide application for the environment, this method is still used alone or in combination with other control agents against numerous fruit fly species. These chemicals have been reported as effective compounds against many species of fruit fly (Mahat and Drew, 2012). Insecticides commonly used include a wide range of carbamates, organophosphates, and synthetic pyrethroids and most recently a new chemical class that contains systemic insecticides, chloronicotinyl (Ekesi *et al.,* 2007). They kill adults' flies on contact and may penetrate fruits to kill eggs and larvae. For instance, diazinon reduced emergence of *C. capitata*, *B. cucurbitae* and *B. dorsalis* considerably when drenched on soil (Stark *et al.,* 2013).

Also, some insecticides have successfully contributed to reduce populations of some species of Tephritidae such as *Bactrocera tryoni, B. cucurbitae, Ceratitis* sp. when combined with other control agents such as traps and attractants and sterile insect technique (Allwood *et al.*, 2002; Reynolds *et al.,* 2018). The spray is on calendar basis, starting when the fruits are at early stage until one to two weeks before fruits are harvested. It requires massive sprays to prevent damage during harvest leading to high pesticides residues. In addition, chemical insecticides adversely affect biological control agents and pollinators that abound in the agro-ecosystems (Ekesi *et al.,* 2007). Various lures including Methyl Eugenol (Plate 2.5), Cue-lure, TriMed-lure, etc. are used as males attractants (Vargas *et al.*, 2012; Tan *et al.*, 2014). Some commercial food attractants (CeraTrap, Captor + Borax, BioLure) may also be used (García-Martínez *et al.,* 2018) such as: GF-120 bait, mixture of sugar, vinegar and wine as well as Torula yeast (Zhou *et al.,* 2012).

2.6.2 Cultural practices to control Fruit Flies

There are several traditional procedures which have been used successfully to reduce fly infestation in farms. Orchard sanitation consists of collecting and destroying infested fruits present on the tree to reduce fruit fly populations. Even though this practice is labour intensive, its effectiveness is based on the frequency of collection all along the entire season (Ekesi and Billah, 2006). Infested fruits with fruit fly larvae can

Plate 2.5. Use of Methyl Eugenol as attractant for the mass trapping of *Bactrocera dorsalis* in Benin Republic

Source: de Souza *et al.* (2016)

also be collected in an Augmentorium made by researchers at USDA ARS in Hawaii. This is made up of fine mesh of about 3 mm^2 which prevents emerging fruit flies from leaving the device giving the chance to beneficial parasitoids to getaway (Vargas *et al.,* 2015).

Infested fruits are one of the potential infection sources in the following season which could lead to increased fly populations in the field. They should be collected, placed into plastic bags and exposed to the sun (Badii *et al.,* 2015). Mature fruits can also be bagged to prevent them from infestation by fruit flies. In addition, some fruits which are not infested when they are 100% green (papaya, banana and sapodilla) can be harvested early to evade fruit fly infestation (Minas *et al.,* 2016). Some works and reviews have shown that cultural methods prevent adult emergence from the soil (Heve *et al.,* 2017; Hasyim *et al.,* 2018).

2.6.3 Biological control of Fruit Flies

The use of parasitoids and predators as natural enemies for the reduction of fruit flies is economically and ecologically safe (Ekesi *et al.,* 2010). Considerable and extensive research works have been conducted to obtain effective parasitoids for biocontrol of fruit flies worldwide. Majority of these parasitoids belong to Braconidae' family. However, few species are known to be very effective against majority of fruit fly'species. For example, *Fopius arisanus* (Plate 2.6a), *Fopius vandenboschi*, *Diachasmimorpha longicaudata* (Plate 2.6b) and *Psyttalia incisi* have become established as the oriental fruit fly. In Hawaii, *F. arisanus* produced about 70% parasitization of *B. dorsalis* in guava (Peters, 1997). Several authors described various strategies, including classical biological control, inoculation biological control, inundation biological control and conservation biological control for introducing bio-control agents within insect control programmes (Garcia and Ricalde, 2013; Sarwar, 2015). For instance, conservation biological control with weaver ants *Oecophylla longinoda* (Hymenoptera: Formicidae) in tree crops with important economic value for farmers in Africa and Asia has been well developed (Van Mele, 2008; Sinzogan *et al.,* 2008). *Oecophylla longinoda* exhibit a highly organized predatory behaviour within an occupied area by a given colony. In Benin, studies have shown the benefits of those ants. It was demonstrated that the higher the number of *O. longinoda* in a mango orchards, the lower the number of damaged fruits recorded (Plate 2.6c) (Van Mele *et*

al., 2008). Nonetheless, the amount of weaver ants per tree highly influenced the damages and losses in fruit flies' infestation (Adandonon *et al.,* 2009). Recently, introducing insect enemies such as parasitoids, predators and entomopathogens (Plate 2.6d) in agricultural system has been receiving increasing attention. Biological control is an economical method and poses less threat than pesticides to humans and the environment (Rizvi *et al.,* 2015). This has encouraged many researchers to evaluate different biological control agents as a replacement to pesticides against several harmful insect including fruit flies (Rouse *et al.,* 2008). The microbial agent *Metarhizium anisopliae* has been effectively used for reducing the menace of fruit flies on Citrus and mango (Thaochan and Ngampongsai, 2018). It is known that *Beauveria bassiana* and its related species have great potential for control of fruit flies (Al-Masri, 2015; Potrich *et al.,* 2018).

2.6.4 Integrated Fruit Fly Management

Fruit fly management can be divided in three main categories: chemical, cultural and biological. Integrated fruit fly management includes the use of several adopted controls measures for suppressing fruit fly populations (Vargas *et al.,* 2015). Each control measure taken individually can reduce a fly population to low levels. However, incorporation of more than one control measure together in an integrated fly management programme has been proved to achieve better control with potential advantages of increasing fruit yield and making the environment safer (Ruiu, 2015). Jang *et al.* (2008), reported that 90 % of melon fly *Bactrocera cucurbitae* was controlled after using bait and trapping systems in incorporation with other control agents. Recently, bait stations based on several attractants have been integrated into sterile insect programmes for many species of Tephritids flies including *Dacus* genus showing effectiveness in monitoring and control (Barclay *et al.,* 2014; Suckling *et al.,* 2014).

2.7 Use of Entomopathogenic Fungi in Fruit Flies' Management

Entomopathogenic fungi are living microorganisms which infect insects causing disease to the hosts in appropriate conditions. They are distributed in different natural habitats and may cause death of a wide range of hosts (Sujeetha and Sahayaraj, 2014). About 750 species from 100 genera of fungi are considered and documented as insect pathogens (Leger and Wang, 2010). Only few are used as pathogens against insect pest

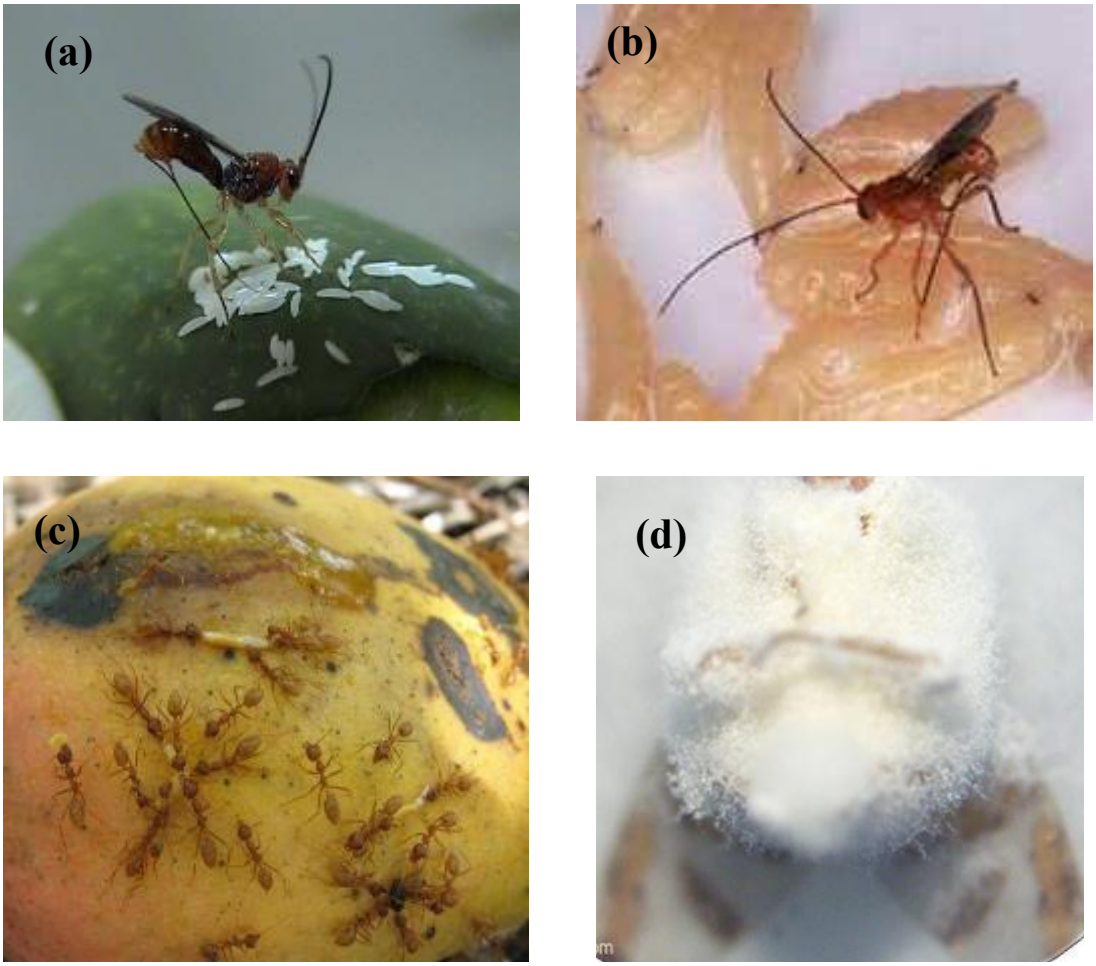


Plate 2.6. Some biological control agents used fruit flies' management program

a = Parasitoid, *Fopius arisanus* depositing eggs inside fruit fly eggs (Vargas *et al.,* 2000)

b = Parasitoid, *Diaschamimorpha longicodata* parasitizing larvae of *Anastrepha suspensa* (Weems and Hepper, 2001)

c = Predators, *Oecophylla longinoda* preying on fruit fly larvae (Vayssières *et al.,* 2013)

d = Cadavers of adult infected by an entomopathogenic fungi (Qazzaz *et al.,* 2015)

(Roberts and Hajek, 1992). Species belonging to *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria* fungi are commercially produced as bio-pesticides against several pests (Vega *et al.,* 2009). The genera of Hypocreales such as *Beauveria*, *Metarhizium*, *Paecilomyces* and *Lecanicillium* can release toxins leading to death of the host (Arora and Shera, 2014). Insect pathogenic fungi are generally safe to humans and the environment which make them most attractive as biological control agents (Zimmermann, 2007). This has prompted many investigations of their pathogenicity against major economically important insect pests (Carrillo *et al.*, 2014; Kassab *et al.*, 2015).

2.7.1 Naturally occurring entomopathogenic fungi

Natural infection rates in fruit fly is usually low with an epizootic of fungal diseases uncommon. Natural infection of adult of *Ceratitis capitata* by *Entomophthora muscae* and *E. schizophorae* has been reported in Israel (Uziel *et al.,* 2003). *Stigmatomyces* spp. was found to naturally occur on *Anastrepha striata* at different elevations in two neotropical forest environments of Costa Rica and Guatemala. The fruit flies were observed to carry perithecia of *Stigmatomyces* species on the insects' legs and either on the ventral part of the thorax for the males; while spores are attached to both sides of the notum and or the dorsal base of the abdomen (Hedström, 1994). In Costa Rica, these fungi occurred more frequently on *A. striata* from non-seasonal forest environments of the Caribbean slopes. The fungus *Scopulariopsis brevicaulis* has also been reported to attack pupae of rose fruit fly, *Rhagoletis alternata*. *Isaria fumosorosea* and *Beauveria bassiana* were reportedly isolated from *Anastrepha* sp. and *C. capitata*, respectively (Castillo *et al.,* 2000). Also, *B. bassiana* and three wild-type fungi (*Mucor hiemalis*, *Penicillium aurantiogriseum* and *Penicillium chrysogenum*) were isolated from *Bactrocera oleae* in Greece (Kostantonopolou. and Mazomenos, 2005).

2.7.2 The entomopathogenic Deuteromycetes, *Beauveria bassiana* (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff)

Beauveria bassiana and *M. anisopliae* pertain to the division Ascomita, class Deuteromycetes (Hyphomycetes), order Hypocreales and family Clavicipitaceae (McCoy *et al.,* 1988). They are known to cause death in more than 220 insect species (Scholte *et al.*, 2003). These fungi are distributed all over the world and can be isolated

from insects and soils (Roy and Penny, 2006). *Beauveria bassiana* and *M. anisopliae* produce reproductive spores. The infected host is killed due to a number of mechanisms harming the cuticle (Clarkson and Charnley, 1996). Entomopathogens are being commercialized and mostly used as to suppress pests of agricultural crops and some vectors of disease including flies and cockroaches.

2.7.3 Mode of action of an entomopathogenic Deuteromycetes fungi

The spores of fungi sticks to the insect inter-segmental folds by tarsal contact (Scholte *et al.,* 2003a; Lacey and Shapiro-Ilan, 2008). Conidia are attached to the cuticle of the host by producing the germ tube (Charnley, 1989). Spores germination depends on surrounding conditions such as temperature, humidity, host susceptibility amongst others. Conidia that germinated form an appressorium from where a peg-like hypha grows that, enters the host (St. Leger *et al.,* 1991). Disease is produced in the host' haemocoel (Charnley, 1989). Fungal pathogens then release a toxin inside the host inducing its death (Strasser *et al.,* 2000). It takes about 2 to 15 days after infection for an infected insect to die contingent upon the fungi strain and species as well as the attributes of the target host (Ferron *et al.,* 1991; Boucias *et al.*, 1995). The spores spread by direct contact to other hosts or by other factors such as wind causing new infection to susceptible hosts (Inglis *et al.,* 20010). Thereafter, the fungus goes to the previous hyphal stage and favorable conditions bring out the subsequent fungal proliferation on the cadaver' surface. A new external and infective conidial saprophyte is then produced (Fargues and Luz, 2000). Other individuals of same target species got easily infected by horizontal transmission from fungal grown on cadavers (Quesada-Moraga *et al.,* 2004; Charnley and Collins, 2007).

2.7.4 Host range of fungal species

Beauveria bassiana and *M. anisopliae* have a large range of host. However, *M. anisopliae* is specific as compared to *B. bassiana.* Some strains within a species are more restricted and sometimes significant differences are observed between species (Furlong and Pell, 2005). *Metarhizium anisopliae* var. *anisopliae* can infect most classes of insects including arachnids (Scholte *et al.,* 2003a). Under laboratory conditions, isolates are more specific as compared to field conditions (Prior *et al.,* 1995). Hence, the geographic occurrence of the fungus is a major factor to be

considered in host preference. The virulence of entomopathogenic fungi differ within species and isolates (Bateman *et al.,* 1996).

2.7.5 Factors influencing fungal pathogenicity

Various factors which are related to the insect host, pathogen and environment have different impacts on the pathogenicity and spread of the disease. For example, soil moisture, temperature and humidity are key factors influencing survival of fungal pathogens (Fargues and Luz, 2000; Filotas and Hajek, 2004; Fuxa and Richter, 2004). In general, germination, growth and persistence lays on abiotic factors, as well as the physiological aspect of the host insect (Wraight *et al.,* 2001; Scholte *et al.,* 2004). The virulence of an entomopathogenic fungus strongly depends on temperature, humidity and light (Sun *et al.,* 2003). The favorable temperature is between 20-30ºC, however *Beauveria bassiana* performs well at 25 ºC while the best temperature for *M. anisopliae* ranged between 27 and 28ºC (Fargues and Luz, 2000; Rai *et al.,* 2003). Temperature can influence the speed and rate of the fungal infection (Inglis *et al.*, 2001). Ambient temperature strongly affects the efficacy of entomopathogenic fungi, and, due to the considerable differences observed between isolates at certain temperatures, is often argued to be the most important environmental factor when selecting isolates to develop as mycoinsecticides (Bugeme *et al,.* 2008; Jaronski, 2010). *Beauveria* response to temperature stresses through sporulation, germination, growth, and virulence over the previous four decades making it the most frequently tested environmental factor in *Beauveria* ecology (Ekesi *et al.,* 1999; Bugeme *et al.,* 2008). Despite a wide range of arthropod taxa tested for susceptibility to *B. bassiana,* including Acarina (Bugeme *et al.,* 2008), Thysanoptera (Ekesi *et al.,* 1999), Hemiptera (Yeo *et al.,* 2003), and so one, minimal differences between optimal temperatures for virulence have been observed, indicating that thermal thresholds for virulence are similar across isolates.

Relative humidity (RH) and substrate water potential also limit survival of entompathogenic fungi because high water availability is required for germination (Devi *et al.,* 2005). The availability of water regulates conidiogenesis on cadavers which have suffered from mycosis (Inglis *et al.,* 2001). Relatively low virulence is likely in environments with a relatively low RH ranging from 46 – 53% (Lord, 2011).

As with thermoregulation in insects and shade provided by plants to reduce UV harm, boundary layers containing high moisture surround vegetation and arthropod exoskeletons which can allow for *B. bassiana* to persist in arid environments. Similarly, rainfall plays a role in conidial dispersal and the ability of entomopathogenic fungi to survive in varying environments, though it can also aid in dislodging *B. bassiana* from its host in many microhabitats (Inglis *et al.,* 1999).

Ultraviolet light, particularly UV-A and UV-B, is another imiting factor responsible for the short persistence of fungal entomopathogens in natural and managed environments (Jaronski, 2010). Like with temperature stress, UV radiation leads to oxidative stress in *B. bassiana* (Lovett and St. Leger, 2018). Irradiance from the UV-A component of solar light (320 – 400 nm) can lead to conidial death and delayed sporulation (Braga *et al.,* 2001). Additionally, the UV-B component (280 – 320 nm), while only accounting for around 5% of total solar irradiance, typically causes more tissue damage to fungal entomopathogens than UV-A (Moore *et al.,* 1993; Inglis *et al.,* 2001). UV exposure inhibits fungal growth, sporulation, germination, and pathogenicity for practically every isolate examined and is often considered the most limiting environmental factor in field applications of B. bassiana (Fernandes *et al.,* 2015). Tropical isolates can tolerate more light exposure than non-tropical isolates, indicating that converse to thermal tolerances, geographic range does potentially correlate to tolerance of solar Radiation (Fernandes *et al.,* 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Sites

This study was conducted in four institutions namely: University of Ibadan in Nigeria; International Institute of Tropical Agriculture (IITA); University of Abomey-Calavi (UAC) and Directorate of Vegetable Production (DPV) in Benin Republic. Field investigations regarding the search for new entomopathogenic fungi were done in selected vegetable plots at Porto-Novo and Zinvié in Republic of Benin. Isolation of fungi was carried out at the Phytopathology laboratory of the Directorate of Vegetable Production situated at Porto-Novo. The biology, ecology and management of the fruit flies were conducted at the Insect Pathology Laboratory of IITA located at Abomey-Calavi, near Cotonou, Benin Republic; where fungal isolates of the two genera of entomopathogenic fungi were maintained. Initial population of fruits fly species were provided by the Laboratory of Agricultural Entomology (UAC-Benin Republic). Mass rearing and maintenance of fruit fly species were done at the Entomology Laboratory, IITA-Benin. The field experiments were conducted at IITA-Benin where *Citrullus lanatus* (watermelon) were grown.

3.2 Establishment of Insects Culture

Colonies of *D. vertebratus* and *Z. cucurbitae* were established from pupae collected from infested *Cucurbita pepo* (zucchini) at the fruit fly section of the Entomology Laboratory of IITA-Benin Republic. Upon emergence, 100 males and 100 females of each fly were placed in different Plexiglas cages of 20 x 20 x 20 cm, having a one side with sleeve attached through which manipulation was done. Flies were mass produced continuously for five generations on the fruit host for laboratory adaptation. Adults were supplied with brown sugar and yeast mixed at 3:1 ratio as adapted from Ekesi *et al.* (2011). Water was provided through entomological vial soaked with cotton wool.

Adults of both sexes of 10-15 days post emergence were used to initiate a colony of flies. Infested fruits were removed after 72 hours of infestation and incubated in small plastic box of 1500 cm^3 containing wire-netting and sterilized sand for pupation. Muslin cloth was used to cover the whole incubation unit which was cloth maintained with an elastic band. Pupae were collected after 10 days and kept for adult emergence. From these cultures, third instar larvae, pupae and mature gravid females were selected all along the experiments. The temperature and relative humidity (RH) of the rearing laboratory were 26 ± 1°C, 70 ± 5% RH, with a photoperiod of 12:12 hours (L:D).

3.3 Sources of Fungal Isolates, Bio-insecticide and Chemical Pesticide

3.3.1 Source of fungal isolates

Isolates of fungi were obtained from the fungal bank of the Pathology Laboratory of IITA-Benin. Six isolates of *B. bassiana* (Bb13, Bb14, Bb337, Bb338, Bb339, Bb353) and one isolate of *M. anisopliae* (Ma31) were selected from 45 isolates based on conidia germination test. They were originally stored on silica gel or in powder form at IITA, Benin Republic and belong to two genera, *Metarhizium* and *Beauveria* as representatives of each species (Table 3.1). One isolate of *M. anisopliae* was collected from the Plant Protection Service at Dakar in Senegal. The fungi were initially produced on plated Potato Dextrose Agar (PDA) medium and incubated for 15 days. Conidia of germinated isolates were subcultured on PDA with ten Petri dishes each and harvested for conditioning test. Experimental conditions for all fungal isolates inside the incubation room were the same as described in section 3,2.

3.3.2 Bio-insecticide and chemical pesticide

The commercial bio-insecticide was formulated with *Metarhizium anisopliae* isolate ICIPE-69 at a recommended rate of 200 mL/ha (1 x 10^9 CFU/mL). The chemical pesticide namely K-optimal is used by the farmers to control insects infesting watermelon in the field in Benin Republic. The active ingredients of K-optimal® are Lambda-Cyhalothrin (15g/L) and Acetamiprid (20g/L) at recommended rate of 1000 mL/ha. Both products were used for field experiment.

Table 3.1. Sources and isolates of entomopathogenic fungi used

Accession number	Pathogen	Host Family	Host Order	Host Species	Country
13	*B. bassiana*	Noctuidae	Lepidoptera	*Eldana saccharina*	Benin
14	*B. bassiana*	Noctuidae	Lepidoptera	*Eldana saccharina*	Benin
337	*B. bassiana*	Pentatomidae	Hemiptera	*Nezara viridula*	Brasil
338	*B. bassiana*	Pentatomidae	Hemiptera	*Oebalus poecilus*	Brasil
339	*B. bassiana*	Coreidae	Hemiptera	*Leptoglossus fulvicornis*	USA
353	*B. bassiana*	Bruchidae	Coleoptera	*Callosobruchus* sp.	Benin
31	*M. anisopliae*	Noctuidae	Lepidoptera	*Sesamia calamistis*	Benin
D	*M. anisopliae*	Tephritidae	Diptera	*Bactrocera dorsalis*	Senegal

3.3.3 Vegetables and fruits used as test crops

Experiments were conducted on eight different natural fruits and vegetable hosts known to be infested by *D. vertebratus* and *Z. cucurbitae.* They include: *Citrullus lanatus* (watermelon), *Carica papaya* (pawpaw), *Cucumis melo* (melon), two species of *Cucumis sativus* (cucumber and pickle) and three varieties of *Cucurbita pepo* (caserta-zucchini, magda-zucchini and fordhook-zucchini) (Plate 3.1). All vegetable fruits were bought at the local market at Porto-Novo in Benin Republic. They were washed and examined with a hand lens magnification for visible signs of oviposition punctures, cleaned and kept for about 3 days in the refrigerator to avoid any external infestation.

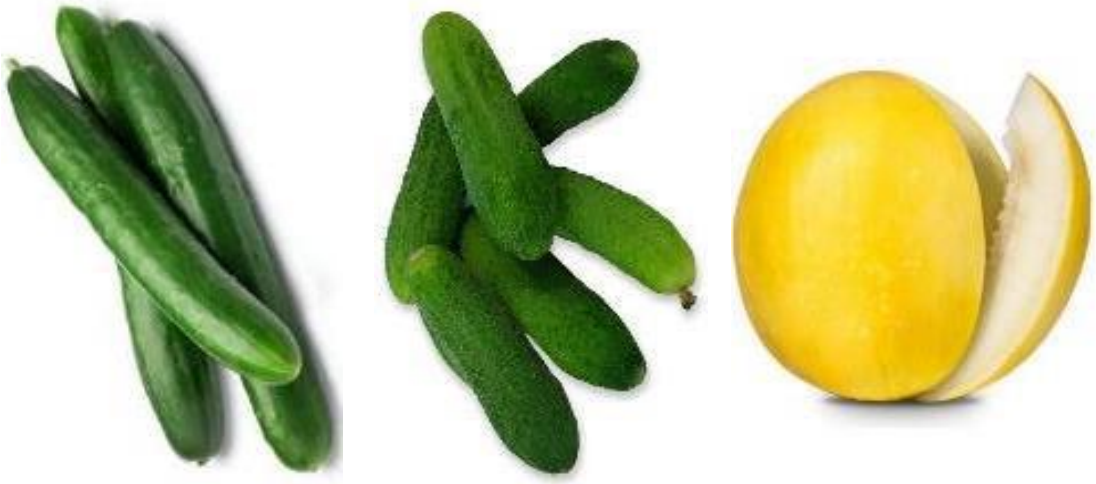
3.4 Locations sampled for soil for growing vegetables to assess the occurrence, abundance and diversity of fungi in Benin Republic

Fungi occurrence, abundance and diversity were assessed from soil samples taken from plots of different vegetable crops at six locations. Three organic farming (Oganla, Ouando and Zinvié) and three conventional farming (Misserete, Akron and Agata) locations were randomly selected for this experiment.

3.4.1 Isolation of indigenous entomopathogenic fungi in vegetable plots through soil sampling in both organic and conventional farming practices

Soil samples were taken from six different crops in three conventional and three organic cucurbits farming practices. The list of vegetable crops and locations are presented in Table 3.2. Samples were taken from the inner 5 - 15 cm layer around the stem of a single plant (Gaddeyya *et al.,* 2012; Chandini and Rajeshwari, 2017). Soil samples were poured in sterilized plastic container of 9 x 6 cm with the lids made of a muslin cloth to facilitate air penetration. All plastic containers were labeled and taken to the laboratory for fungi isolation. All soil samples were maintained in the laboratory at ambient conditions of 28 ± 2°C day and 22 ± 2°C night, 75 ± 5% RH, for further evaluation of abundance and diversity of indigenous fungi with capacity to suppress insect' pests.

Cucurbita pepo var. magda-zucchini | *Cucurbita pepo* var. caserta-zucchini | *Cucurbita pepo* var. fordhook-zucchini



Cucumis sativus (cucumber) | *Cucumis sativus* (pickled-cucumber) | *Cucumis melo* (melon)

Carica papaya var. solo (pawpaw) | *Citrullus lanatus* var, Kaolock (watermelon)

Plate 3.1. Vegetable host fruits used for biological study of fruit fly species

Table 3.2. List of vegetable crops and location of farms in Benin Republic

Type of farming	Location	Type of Crop	Common name
Conventional farming	Akron, Porto-Novo	*Amaranthus viridis*	Amaranth
		Solanum macrocarpon	Egg-plant
		Cucumis sativus	Cucumber
		Vernonia amygdalina	Bitter leaf
		Capsicum annuum	Pepper
		Ocimum gratissimum	Wild basil
	Agata, Porto-Novo	*Cucumis sativus*	Cucumber
		Solanum lycopersicum	Tomato
		Vernonia amygdalina	Bitter-leaf
		Solanum macrocarpon	Egg-plant
		Amaranthus viridis	Amaranth
		Capsicum annuum	Pepper
	Missérété, Porto-Novo	*Citrullus lanatus*	watermelon
		Cucumis sativus	Cucumber
		Vernonia amygdalina	Bitter leaf
		Amaranthus viridis	Amaranth
		Capsicum annuum	Pepper
		Solanum lycopersicum	Tomato
Organic farming	Directorate of vegetable production, Porto-Novo	*Amaranthus viridis*	Amaranth
		Solanum macrocarpon	Egg-plant
		Citrullus lanatus	Watermelon
		Cucumis sativus	Cucumber
		Capsicum annuum	Pepper
		Amaranthus viridis	Amaranth
	Horticultural Centre Porto-Novo	*Solanum macrocarpon*	Egg-plant
		Daucus carota	Carrot
		Capsicum annuum group	Sweet pepper
		Capsicum annuum	Pepper
		Lactuca sativa	Lettuce
		Amaranthus viridis	Amaranth
	Sita-Toundra, Zinvié	*Amaranthus viridis*	Amaranth
		Lactuca sativa	Lettuce
		Solanum nigrum	Morelle noire
		Celosia sp.	Celosia
		Petroselinum crispum	Parsley
		Vernonia amygdalina	Bitter leaf

3.4.2 Assessment of efficacy of insect bait method for field collection of entomopathogens

Sub-samples of 100 g taken from each collected soil samples was poured in a graduated plastic container (7 cm x 4.5 cm) (Plate 3.2a). The samples were air-dried and sterile distilled water was added to 30% water holding capacity (Meyling and Eilenberg, 2007). The insect bait method originally developed to isolate entomopathogenic nematodes and fungi from soil samples was adopted (Zimmerman, 1986). Ten third-instar larvae of *D. vertebratus* were introduced into sample using a camel hair brush and arranged in incubators at 26 - 27 ^{o}C in the dark (Plate 3.2b). Larvae were examined every day for five days. Dead larvae were then collected from soil substrates and deposited in Petri dishes. Pupae were decontaminated in 70% ethanol and cleaned in three washing of sterile distilled water to remove external pathogens (Plate 3.2c). Dead larvae and pupae were put on sterile Whatman No 5-filter paper placed on PDA supplemented with Streptomycin preventing growth of bacteria (Plate 3.2d). Plates were sealed with para-film and kept in store room. Evidence of fungal growth was checked five days post incubation on the dead pupae in Petri dishes.

3.4.3 Assessment of efficacy of serial soil dilution method for fungi isolation

Serial soil dilution was also used to extract fungi from soil samples of both organic and conventional farming practices. Ten grams of each soil was poured into a 10 mL graduated cylinder. Sterile distilled water of 90 mL was added to the soil and mixed thoroughly to increase the chances of isolating other fungi that might be under-represented. Serial dilutions (10^{-1} to 10^{-5} mL) of each suspension were made (Waksman, 1994). Followed the procedures adapted by Gaddeyya *et al.* (2012), one millilitre of soil suspension of each concentration (10^{-3}, 10^{-4} and 10^{-5} mL) was dispersed onto cooled Potato Dextrose Agar added with 1% Streptomycin solution to prevent growth of bacteria and previously sterilized at 121°C for 20 min. Each dilution level was replicated four times. Sealed plates were stored at 25°C for a week (Omoloye *et al.,* 2015). Identification of different fungi species were done and pure cultures were made (Mohammadbeigi and Port, 2013).

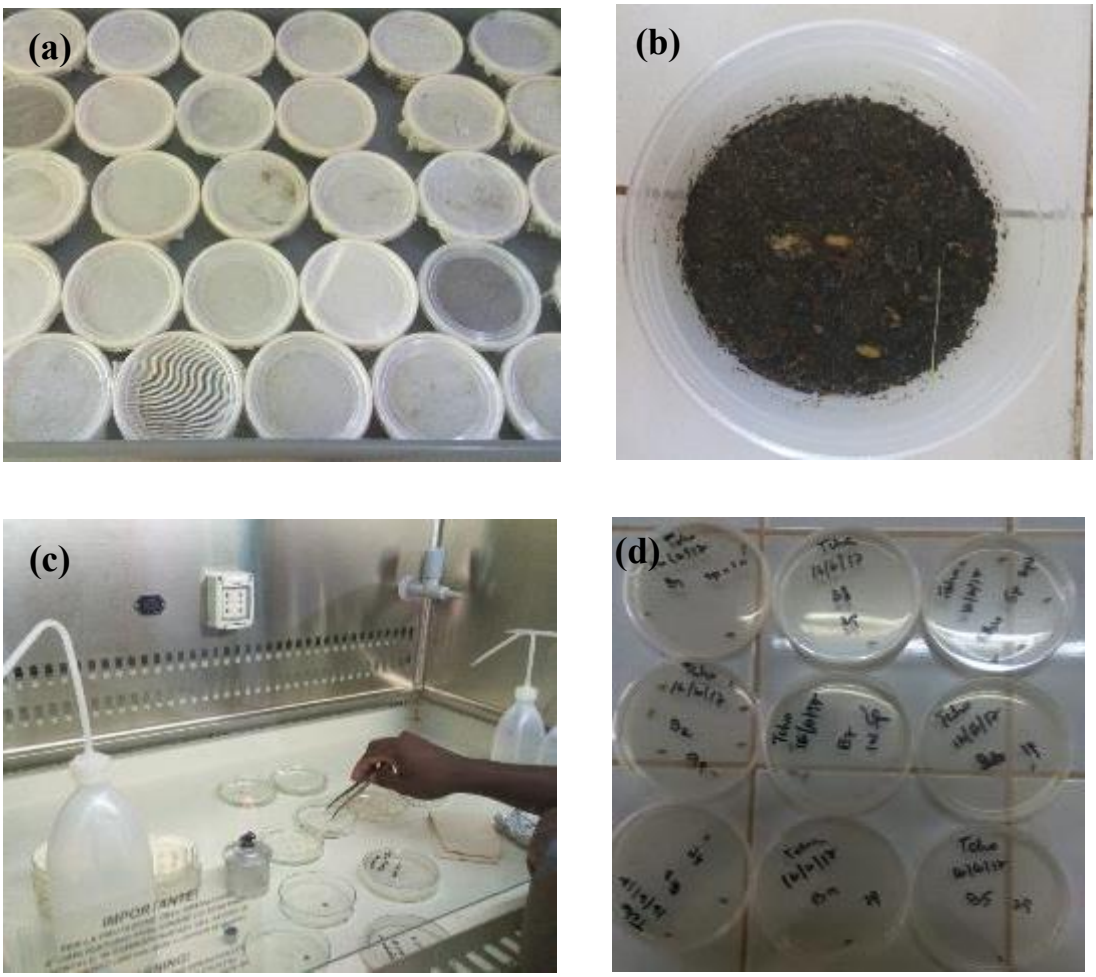


Plate 3.2. Layout and procedures for collection of fungi using insect bait method

a = Soil sub-sample of 100 g in plastic containers

b = Soil sample containing pupae of *D. vertebratus*

c = Sterilization of pupae collected from soil sample

d = Sterilized pupae plated on PDA

3.4.4 Identification of fungi isolated from different soils

Fungi species were classified by preparing slides mounting using lacto-phenol cotton blue method. Fungi were examined using stereo-binocular microscope. Conidia, conidiophores and arrangement of spores were observed microscopically. Spores morphology, character and structure were used to characterize each species (Nagamani *et al,* 2006).

3.4.5 Evaluation of the efficacy of fungal species isolated from different soil samples on *Zeugodacus cucurbitae* and *Dacus vertebratus*

This bio-assay was run in a Completely Randomized Design (CRD) under the same laboratory conditions. Five replicates were made per fruit fly species. Ten third instar larvae of 5 to 7 days old of *D. vertebratus* and *Z. cucurbitae* were used per replicate. Conidial suspensions of isolated fungi and bacterium (*Aspergillus niger, Fusarium verticillioides*, *Botryotricum* sp. and an antibiotic, *Streptomycin* sp.) were prepared for evaluation of mortality in *D. vertebratus* and *Z. cucurbitae*. Suspensions of each fungal and bacterium species were applied topically at a rate of 10^{10} conidial/mL and 10^{9} CFU/mL for the bacterium onto the larvae initially placed in sterile plastic box of 3.5 x 4 cm. Larvae in control was treated with Tween 80 (0.1%) solution. All dead host instars were put on a sterile Whatman No 5 filter paper in Petri dishes and humidified with Tween 80 solution. Petri dishes were sealed and maintained in a store room. Cadavers were checked daily for two weeks for expansion of fungi on the exterior part of the infected larvae, pupae and adults. The number of enclosed pupae and /or adults from treated larvae and pupae was also recorded.

3.5 Assessment of biological parameters of *Dacus vertebratus* and *Zeugodacus cucurbitae* infesting *Citrullus lanatus*

The biology of *Dacus vertebratus* and *Zeugodacus cucurbitae* with regard to their life cycle were assessed under laboratory conditions. This experiment was carried out using the Complete Randomized Design (CRD) under a controlled room of 26 ± 1°C and 70 ± 5% RH, and a photoperiod of 12:12hours (L:D).

3.5.1 Collection of eggs of *Dacus vertebratus* and *Zeugodacus cucurbitae*

Eggs were collected using fruits domes as egg collection device as described by Ekesi and Mohamed (2011). Pawpaw fruits were divided equally. The pulp and the seed of the fruit were extracted and domes were punctured with number (00) entomological

pin. They were placed on Petri dish of 9 cm diameter lined with moistened filter paper. Domes were put inside separate cage containing 200 matured flies of both sexes of 10 days old for *D. vertebratus* and *Z. cucurbitae.* Pawpaw domes were left for 10 hours inside the different cages for infestation. Infested domes were removed from the different cages. Eggs were sorted out by washing them with water from the oviposition devices (domes). Freshly collected eggs were counted under stereo-microscope and used to measure several parameters including: egg hatch, percentage pupal recovery, adult emergence, sex-ratio, survival of immature instars of *D. vertebratus* and *Z. cucurbitae.*

3.5.2 Determination of each immature instars duration of *Zeugodacus cucurbitae* and *Dacus vertebratus*

Development time of each immature instars of *Z. cucurbitae* and *D. vertebratus* were determined from 100 eggs. Eggs were collected following the procedures described in section 3.5.1. A cohort of 100 fresh eggs were prepared per fruit fly species and replicated ten times. Eggs hatching were observed under binocular microscope and egg incubation period was recorded. Newly emerged larvae (first instar) were placed onto a fruit of *Cucurbita pepo* var. magda-zucchini of 70 g in plastic containers of 450 m^3. Each host instars duration (eggs, larvae, pupae and adult) were observed accordingly. A cohort was gathered in the middle of the period between the first and last individual that reached a specified instar (Vayssières *et al.,* 2008). Larval instars were separated by looking at their body size, structure and colour (White and Elson-Harris 1992). Third instar larvae were placed thereafter in plastic container with sterilized sand for pupation. Newly formed pupae were recovered by sieving sand. Pupae were examined every 24 h for one week post collection. Pupae were placed in a Plexiglas cage and adults' emergence were recorded two times a day. The life cycle duration of *Z. cucurbitae* and *D. vertebratus* was then established.

3.5.3 Establishment of survival rate of immature instars of *Zeugodacus cucurbitae* and *Dacus vertebratus*

Cohort of 100 eggs of *Z. cucurbitae* and *D. vertebratus* were extracted upon procedures described in section 3.5.2 (Vayssières *et al.,* 2008). Survival rates were recorded for each host instar development of both fruits flies. For each fruit fly species, numbers and percent eggs hatched, larvae, pupae and emerged adults were recorded

daily for 3, 10, and 20 days respectively. The survival rate was determined by calculating the percentage of individuals in a given stage that successfully emerged into a subsequent stage.

3.5.4 Emergence rate and sex-ratio of *Zeugodacus cucurbitae* and *Dacus vertebratus*

Pupae were randomly selected from the largest daily collection. Ten lots of 10 pupae (10 replicates) per fruit fly species and pupae were placed in Petri dishes covered in a Plexiglas cage of 15×15×15 cm until dead of all emerged flies. Emergence rate and sex-ratio was were calculated using the formula:

$$Em\,rate\,(\%) = [(Np - Nnm) \div Np]\,x\,100,$$

Where Em rate = emergence rate; Nnm = Number of non-emerged adults; Np = Number of pupae

$$\text{Sex-ratio} = \text{N-male} \div \text{N-fem}$$

Nmale = Number of males and Nfem = Number of females (Chang, 2009).

3.5.5 Morphometric measurements and morphology of host instars development of *Zeugodacus cucurbita* and *Dacus vertebratus*

The morphological parameters of egg, larvae, pupae and adult were taken using binocular. Body size, body surface sculpturing and colour were used as morphological characters to differentiate larval instars (White and Elson-Harris, 1992). Adults were conserved in 70 % ethanol and then observed under microscope for morphological characters study. Morphometric measurements (length and width) of each host development were done using Electronic Digital Calliper.

3.6 Study the aspects of ecology of *Dacus vertebratus* and *Zeugodacus cucurbitae* in a control environment

The ecology of both fruit fly species in a control environment was assessed with regard to hosts range, temperature and adult artificial diets effect on the development of *Dacus vertebratus* and *Zeugodacus cucurbitae* under laboratory conditions.

3.6.1 Comparative susceptibility of eight vegetable fruits to *Dacus vertebratus*

Susceptibility and suitability of *D. vertebratus* to eight different vegetable host fruits were determined by evaluating the performance of *D. vertebratus* reared in laboratory under two different temperature conditions on these hosts by no-choice tests. Different

vegetables fruits include *Citrullus lanatus* (watermelon), *Carica papaya* (pawpaw), *Cucumis melo* (melon), two varieties of *Cucumis sativus* (cucumber and pickled-cucumber) and three varieties of *Cucurbita pepo* (caserta-zucchini, magda-zucchini and fordhook-zucchini) were used for this experiment.

3.6.1.1 Evaluation of the effect of host ranges and temperature on the total development and longevity of *Dacus vertebratus*

Ten males and ten females of *D. vertebratus* of 10 days old were placed inside plastic boxes of 10 x 6 x 4 cm (Plate 3.3). Adult's diets were made of 1:3 volume of a mixture of yeast and brown sugar (Ekesi *et al.,* 2011). Water was also provided through vials as soaked cotton wool. Each vegetable fruit was weighed and placed in the boxes containing flies. Four replicates were made per vegetable at each temperature of 25 ± 1°C and 31 ± 1°C. Each vegetable was infested for 24 hours by adults flies and incubated in separate plastic bowls of 450 m^3 containing sand and wire-netting and covered with muslin clothes. After two days of incubation, plastics containers were checked twice a day to avoid the unnatural death of larvae due to the host-juice excess Total developmental time of *D. vertebratus* on each vegetable host was established from egg to adult emergence. The longevity of both sexes of *D. vertebratus* during egg laying period was assessed for 40 days and dead flies were recorded daily.

3.6.1.2 Evaluation of the effect of vegetable host and temperature on fecundity of *Dacus vertebratus*

A pair of five males and five females of *D. vertebratus* was placed in eight different Plexiglas cages (15 x 15 x 15 cm) and supplied with adults diets and water. Slices of each vegetable fruit were placed on Petri dishes and introduced inside each cage containing flies. Females laid eggs into slices of vegetable fruits. Each morning, slices of each vegetable fruit were removed and replaced by new fresh slices of vegetable fruits. Eggs were daily recovered and counted from the vegetable slices with a small camel hair brush for 20 days.

3.6.1.3 Evaluation of the effect of vegetable hosts and temperature on pupal production, adult emergence and sex-ratio of *Dacus vertebratus*

Adult of 10 males and 10 females of *D. vertebratus* of 10 days old were placed inside plastic boxes of 10 x 6 x 4 cm and fed as described in section 3.6.1.1. Thirty grams of

Plate 3.3. Layout of the devises containing weighed vegetables fruits infested by *Dacus vertebratus* in the laboratory

each vegetable fruit cut in slices were introduced inside different boxes for 24 hours. Vegetables were removed and kept separately in Petri dish placed on sand inside an incubation unit. Plastics were covered with muslin clothes for pupation. After a week, pupae were collected by sieving the sand; total number of pupae from each vegetable host were counted and subsequent adult emergence was recorded. The number of males and females that emerged from pupae was also recorded and sex-ratio was computed followed the formula described in section 3.5.4.

3.6.2 Effect of two protein-based diets as adult foods for mass rearing of *Dacus vertebratus*

Two protein-based diets were compared under laboratory conditions. One diet was made locally with the mixture of soya-bean and ordinary white sugar and the other made with the mixture of yeast and brown sugar. Each diet was compared at different parental age of 12, 14, 16, 18, 20 and 22 days old and replicated 12 times. The two diets were separately used to feed a couple pair of 100 female and 100 males. Flies were placed into different small Plexiglas cage of 15 x 15 x 15 cm along with three grammes of each diet at 3:1 ratio and water. Diets were replaced each other day throughout the experiment. Cages were placed in the rearing room at 26 ± 1°C and 70 ± 5% RH.

3.6.2.1 Effect of two protein-based diets as adult foods on the total development of *Dacus vertebratus*

Zucchini of 80 g was introduced in different cages containing fruit flies fed with different adult diet (Plate 3.4). Fruits were infested for 24 h and incubated in small plastic box of 450 cm^3 placed in another plastic of 1500 m^3 containing wire-netting and sterilized sand for pupation. Plastic was covered with a muslin material maintained with an elastic band. They were checked daily for the removal of exceeded juice and collection of pupae. Total development time was determined from egg until adult emergence for each adult diet.

3.6.2.2 Effect of two protein-based diets as adult foods on the fecundity of *Dacus vertebratus*

Couple of 5 males and 5 females of *D. vertebratus* were introduced separately in different cage of Plexiglas cages of 15 x 15 x15 cm and fed with different diet (mixture

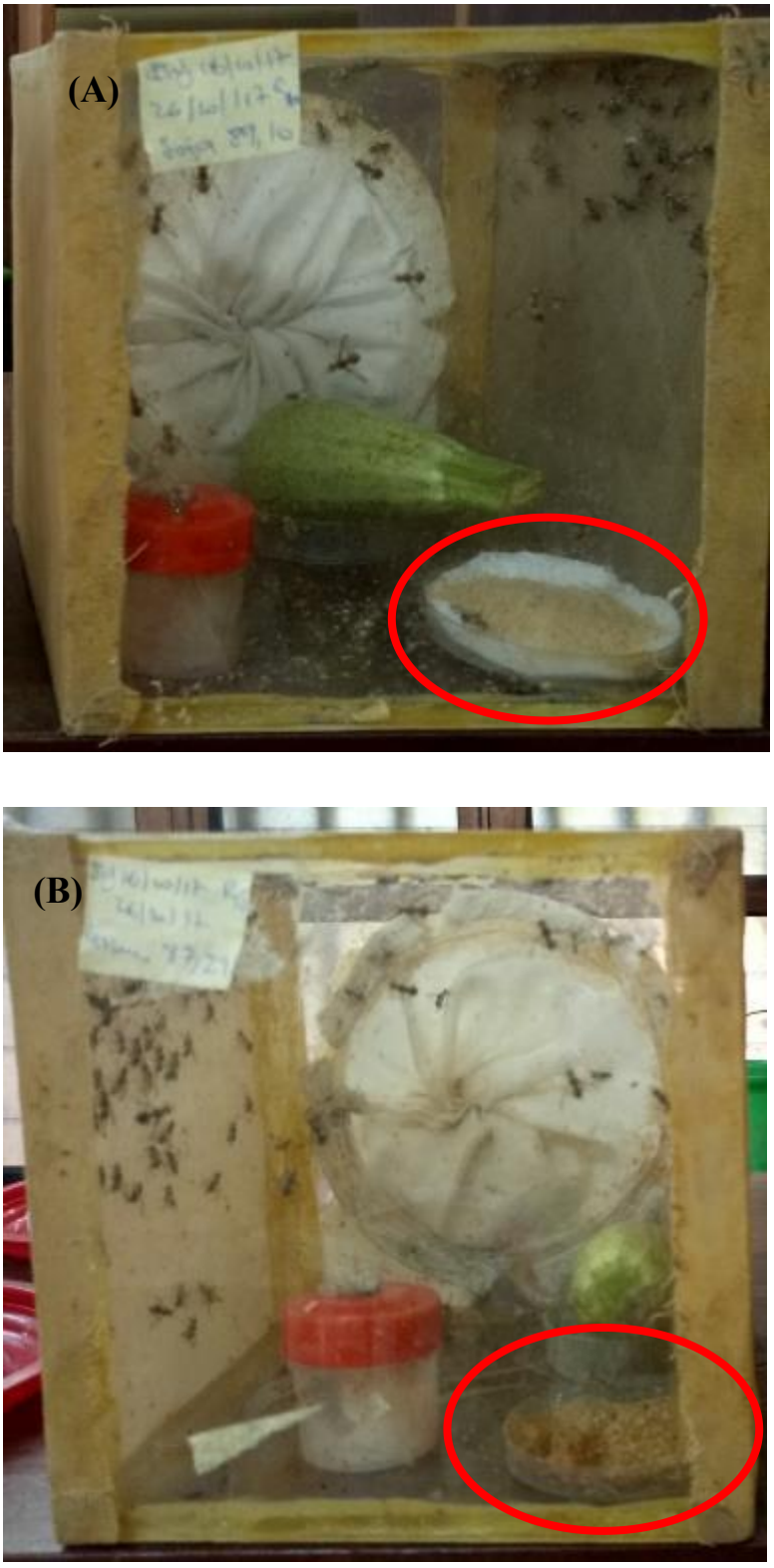


Plate 3.4. Layout of the experiment containing adult flies fed with (A) mixture of yeast and brown sugar and (B) mixture of soybean and white sugar

of soya-bean and ordinary white sugar and mixture of yeast and brown sugar). Slices of *Cucurbita pepo* var. magda-zucchini were introduced inside each cage. Slices covered the surface a 3 cm diameter of a small Petri dish. Females fed with different diets were allowed to lay eggs into slices of vegetable fruit. Each morning, slices of fruits were removed and replaced by new fresh slices of magda-zucchini. Eggs were collected and counted daily for 20 days.

3.6.2.3 Effect of two protein-based diets as adult foods on the number of pupae, adult emergence and sex-ratio of *Dacus vertebratus*

Inside different cage of Plexiglas cages of diameter 15 x 15 x15 cm, pair of 100 males and 100 females of *D. vertebratus* were placed and with different adult diet. Eighty grams of *C. pepo* var. magda-zucchini for 24 hours of infestation by adult flies fed with different adult diet. Infested fruits were incubated separately in plastic container of 450 m^3 and then placed on a layer of sand inside a plastic bowl. Plastic bowls were covered with muslin clothes to permit pupation. Pupae were collected after 10 days by sieving the sand. Pupal production was determined by counting and subsequent emerged adult was recorded. The number of males and females emerged from collected pupae were also recorded and sex-ratio was computed.

3.6.3 Host preference of *Zeugodacus cucurbitae* to three fruits and vegetables fruits in choice and no-choice tests

Host suitability for *Z. cucurbitae* was determined in no choice and choice condition. All flies fed on a mixture of white sugar and soybean at 3:1 ratio. Water was provided through entomological vial soaked with cotton wool. No-choice condition test was conducted on *Citrullus lanatus* (watermelon), *Cucurbita pepo* (magda-zucchini) and *Carica papaya* (pawpaw). For each of the vegetable host fruits, five cages containing a couple of 100 males and 100 females of 12 to 15 days old were prepared. The three vegetables were introduced separately in different small Plexiglas cages of 15 x 15 x 15 cm (Plate 3.5). While in choice condition, a pair of vegetable fruits was introduced into a cage containing a couple of 200 adults (100 females and 100 males). The three fruits and vegetables were also placed inside a cage containing a couple of 150 males and 150 females (Plate 3.6). After 24 hours of infestation, infested fruits were removed and fruits incubated in separate plastics containers of 450 m^3. Each vegetable fruit was repeated five times. Parameters such as larval duration, pupal duration, total

Plate 3.5. Layout of experiment unit under no-choice test

a = *Cucurbita pepo* variety magda-zucchini placed in no choice test

b = *Citrullus lanatus* (watermelon) placed in no choice test

c = *Carica papaya* (pawpaw) placed in no choice test

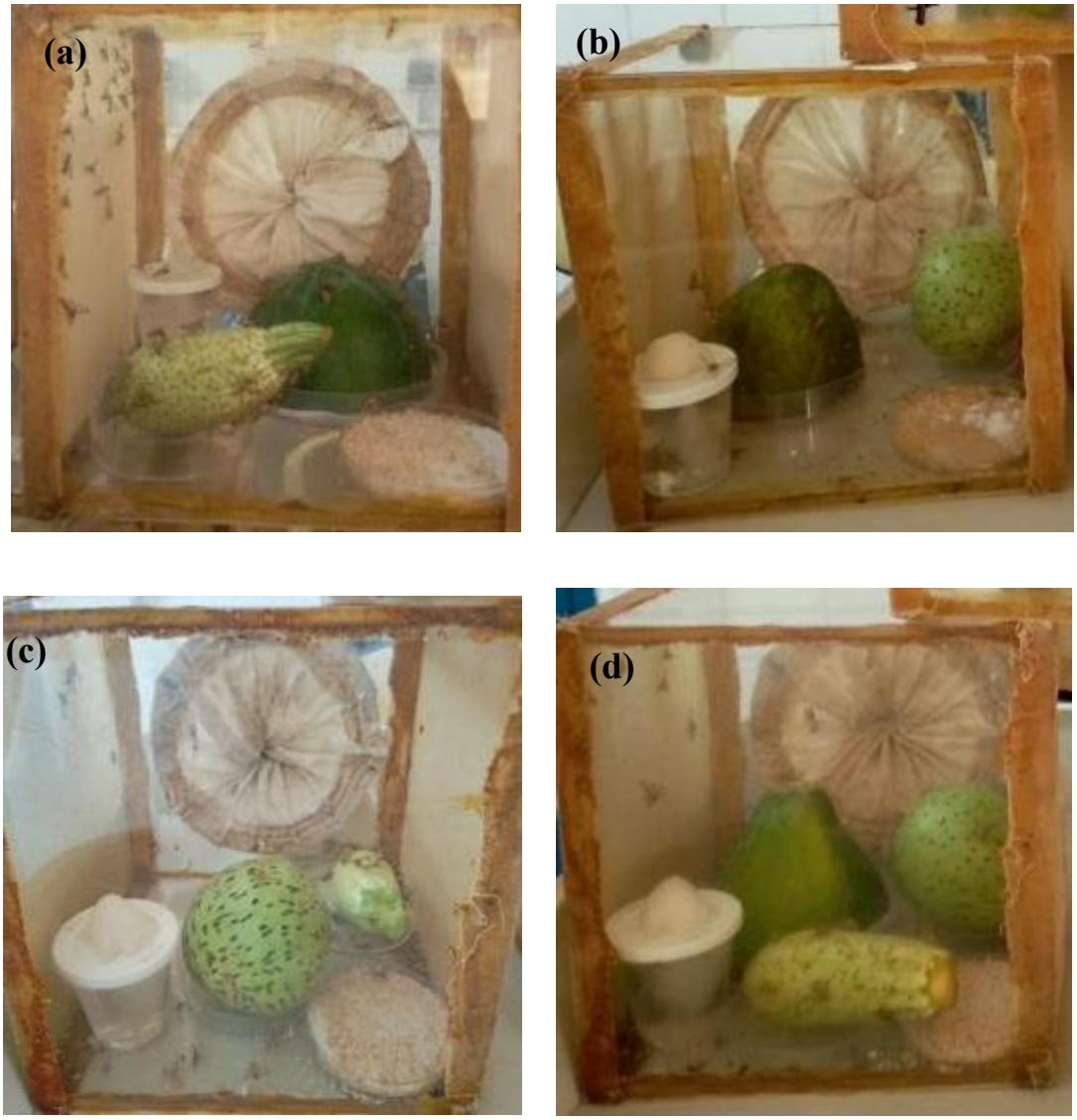


Plate 3.6. Layout of devices under choice test experiment

a = *Cucurbita pepo* paired with *Carica papaya* in choice test

b = *Citrullus lanatus* paired with *Carica papaya* in choice test

b = *Cucurbita pepo* paired with *Citrullus lanatus* in choice test

c = *Citrullus lanatus* paired with *Carica papaya* and *Cucurbita pepo* in choice test

development time, pupal size (length and width), pupal weight, number of produced pupae and subsequent adult emergence rate, flight ability were collected for each host fruits.

3.6.3.1 Effect of vegetable hosts on immature host instars of *Zeugodacus cucurbitae*

A piece of 10 g of each of the vegetable host was infested with 25 eggs of *Z. cucurbitae*. Eggs were collected following standard described above (Ekesi and Mohamed, 2011). Minimum larval duration on each artificial diet was determined using the number of days between eggs hatching and first pupal collection while maximum larval duration was determined using the number of days between eggs hatching and last pupae collected. Pupal duration was determined from first pupal collection until the last adult emergence. The duration of different fly species from from egg to adult was determined.

3.6.3.2 Effect of vegetable hosts on number, weight, morphometrics measurement of pupae of *Zeugodacus cucurbitae*

From each vegetable host fruit, pupae were collected and counted. The total pupal production was recorded. Adult emergence was determined from 10 pupae randomly selected using the formula described in section 3.5.4 (Chang, 2009). The weight, length and width of pupae were recorded two days after pupae collection. Pupal weight was determined from a sample of 10 pupae of *Z. cucurbitae* randomly selected from pupae collected for each vegetable host fruits (20 replicates). Each lot was weighed using Adam Equipment PGW 453i digital Balance (Max 450g, d=0.001g), USA. The length and width were measured however from 20 randomly selected pupae.

3.6.3.3 Effect of vegetable hosts on the infestation of *Zeugodacus cucurbitae*

The infestation was determined on the different host. The vegetable host fruits were weighed (in kg) and the subsequent number of pupae collected from each of them was recorded. The following formula as described previously was used (Cowley *et al.,* 1992) :

$$IR = Np \div Wp$$

Where *IR* = Infestation ; Np = Number of pupae and Wp = Weight pupae (kg).

3.6.3.4 Effect of vegetable hosts on the flying ability (fliers) of *Zeugodacus cucurbitae*

Five males and females emerged five days after pupae collection were transferred separately to the bottom of a three compartments cage of 50 x 20 x 25 cm separated with two large holes to allow flies to move from one compartment to another (Plate 3.7a). The cage was covered with black plastic (Photo 3.7b) except at the top and set up on shelves under the light source of the rearing room. Every 10 minutes, flies that moved from the bottom to one of the two compartments at the top were removed and recorded. A last observation was done 5 hours later, on the remaining flies in the compartment below to identify adults that look normal but do not have flying ability.

3.7 Evaluation of the efficacy of fungi with entomopathogenic potential for control of *Dacus vertebratus* and *Zeugodacus cucurbitae*

The efficacy of entomopathogenic fungi was assessed by screening isolates of entomopathogens. Isolates were produced on PDA for conditioning test on *D. vertebratus* and *Z. cucurbitae* and bioassay. The genotoxicity and pathogenicity of isolates were evaluated through field assessment.

3.7.1 Preliminary viability screening of some existing isolates of *Beauveria bassiana, Metarhizium anisopliae* and *M. flavoviride*

The conidia of isolates of *M. anisopliae* (Ma20, Ma29, Ma31, Ma51, Ma55, Ma60, Ma90, Ma92, Ma341, Ma351, Ma357); *B. bassiana* (Bb8, Bb9, Bb10, Bb13, Bb14, Bb337, Bb338, Bb339, Bb340, Bb353) and *M. flavoviride* (Mf66, Mf59, Mf98, Mf91, Mf190, Mf191, Mf192, Mf141, Mf193, Mf427) were selected and checked for viability. Conidia were stored on silica gel or in powder form. Potato Dextrose Agar medium was prepared at the rate of 39 g per litre amended with an antibiotic, streptomycin (0.05/10 mL) in Petri dishes of 9 cm diameter (Plate 3.8a, Plate 3.8b). Conidia of each isolate of fungi were plated on PDA and each isolate was replicated five times. Plates were incubated for 15 days at 26 ± 1°C and 70% RH, photoperiod: 12:12 hours (L: D). Conidia of germinated isolates were harvested for conditioning test (Plate 3.8c, Plate 3.8 d).

Plate 3.7. Device used to assess the flying ability of *Zeugodaucs cucurbitae*

a = Three compartmented cages separated with two large holes

b = Cage was covered with black polythene material

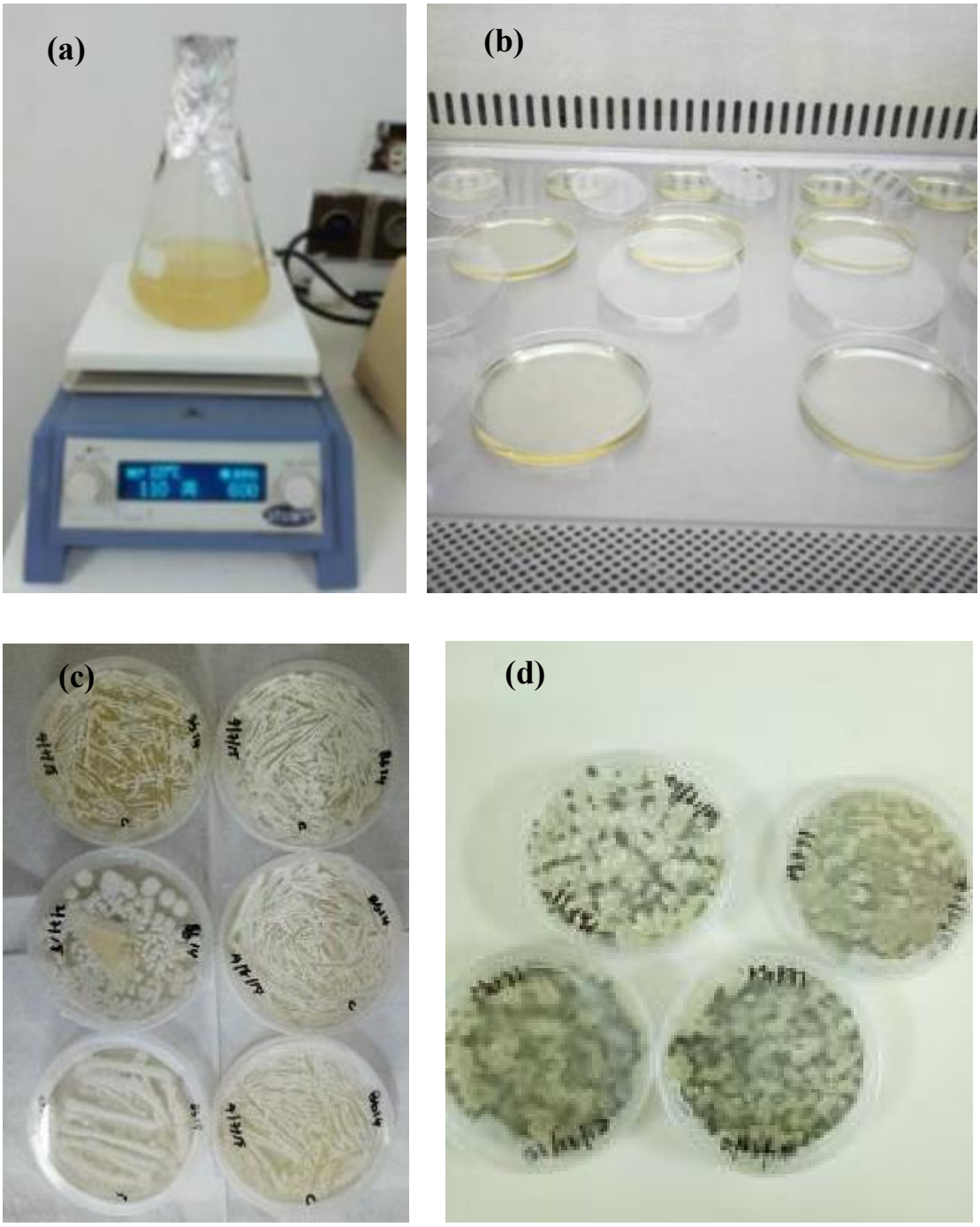


Plate 3.8. Layout and procedures of Potato Dextrose Agar (PDA) preparation and culture of isolate of *Beauveria bassiana* and *Metarhizium anisopliae*

a = PDA mixed with sterilised distilled water in a graduated glass

b = Prepared PDA in Peri dishes + Streptomycin

c = Culture of isolate of *B. bassiana*

d = Culture of isolate of *M. anisopliae*

Using a sterilized spatula, conidia were scratched from the surface of the fungal culture. Five millilitres (5 mL) of sterile Tween 80 (0.1%) solution was poured into the Petri dishes and stirred using magnetic stirrer. The suspension was sieved using a 75-micron filter into a bottle to take off hyphal fragments or mycelial debris. Suspension was vortexed with Fisher Vortex for about two minutes to dissociate conidial clumps. An initial concentration of 10^{10} conidia/mL was prepared per isolate and conidia were quantified with a standard (improved Neubauer) haemocytometer under a phase-contrast microscope. Suspensions were used for conditioning test.

3.7.2 Conditioning of the isolates to *Zeugodacus cucurbitae*

Third instar larvae and fresh pupae of *Z. cucurbitae* were inoculated with the prepared suspensions of conidia of each isolate of *B. bassiana* and *M. anisopliae*. Ten larvae replicated 10 times were placed into different plastic boxes of 3.5 x 4 cm. Using a micropipette, 2 x 10^{-10} µL of each suspension were applied topically onto each larva in the box containing larvae of *Z. cucurbitae*. The boxes with inoculated larvae were incubated at 26°C and larvae were examined daily for 12 days. Dead larvae were placed on a Whatman No 5 filter paper placed inside Petri dish and humidified with Tween 80 solution to check for fungal growth on larvae for 10 days.

3.7.3 Production of new isolates (clones)

From the conditioning test, evidence of fungal growth, that is, dead insects with sporulation was checked. The surface of sporulated cadavers were scraped with entomological needle. Conidia were plated liberally and evenly on PDA following the procedure adapted by Omoloye *et al.* (2015). Petri dishes were covered and incubated for 15 days. Conidia were harvested after incubation time of 15 days for insect bioassay for data on mortality of *Z. cucurbitae.* Surface of new fungal culture of each isolate were scratched and conidia suspensions were made. The number of conidia was quantified using a haemocytometer replicated three times for an accurate precision. Under the microscope, germinating and non-germinating conidia were counted. The germination rate was estimated by counting 100 conidia for each plate and calculated using the formula adapted by Mehinto *et al.* (2014):

$$\%\text{germination} = ([GC + NGC / GC]) \times 100$$

Where *GC* = Germinating Conidia and NGC = Non-Germinating Conidia.

3.7.4 Bioassay studies on virulent isolates of *Beauveria bassiana* and *Metarhizium anisopliae*

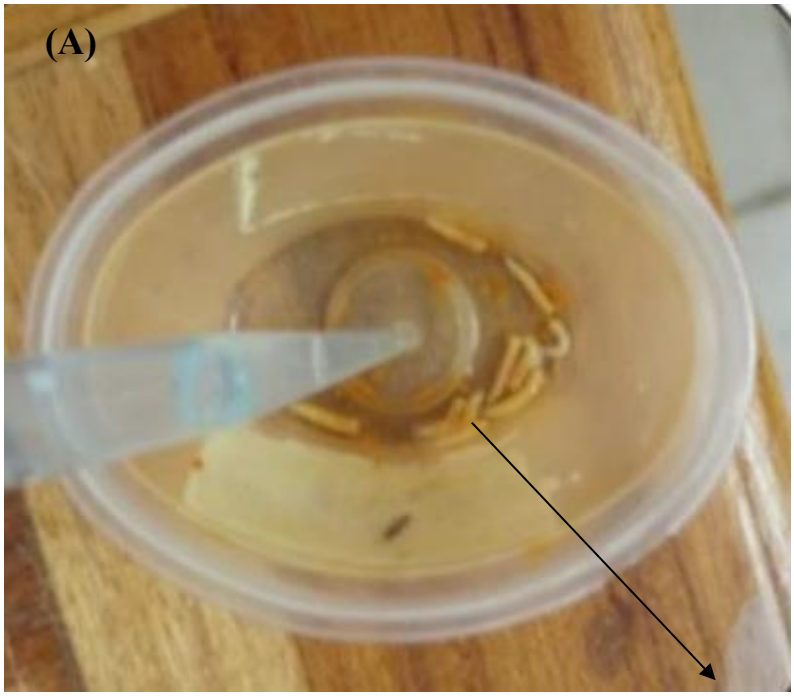
The experiments took place in the same controlled room. Three host stages (larvae, pupae and adults) of *Z. cucurbitae* were used for the experiments. Conidial suspensions of *M. anisopliae* (Ma31) and *B. bassiana* (Bb13; Bb14; Bb337; Bb338, Bb339 and Bb353) for bioassay on mortality of *Z. cucurbitae* were prepared. Different concentrations (10^5 to 10^{10} conidia/mL) of each conidia suspensions were made. For each concentration, five replicates were made. Experiments were run in a Completely Randomized Design (CRD).

Ten third instar larvae (5 to 7 days) and 10 fresh pupae (1 to 3 days old) of *Z. cucurbitae* were collected from artificial infested pawpaw. They were introduced into a sterile plastic box of 3.5 x 4 cm. Each larva (Plate 3.9a) or pupa (plate 3.9b) was inoculated with 2 µL of each concentration (10^5 to 10^{10} conidia/mL) per isolate using a micropipette. A pair of 4 adults of 5 days old of *Z. cucurbitae* was transferred separately into different prepared boxes and sprayed with different concentrations as well. Two controls were set up: untreated groups and groups treated with 0.1% distilled Tween 80. The plastic boxes were maintained at ambient temperature in store room.

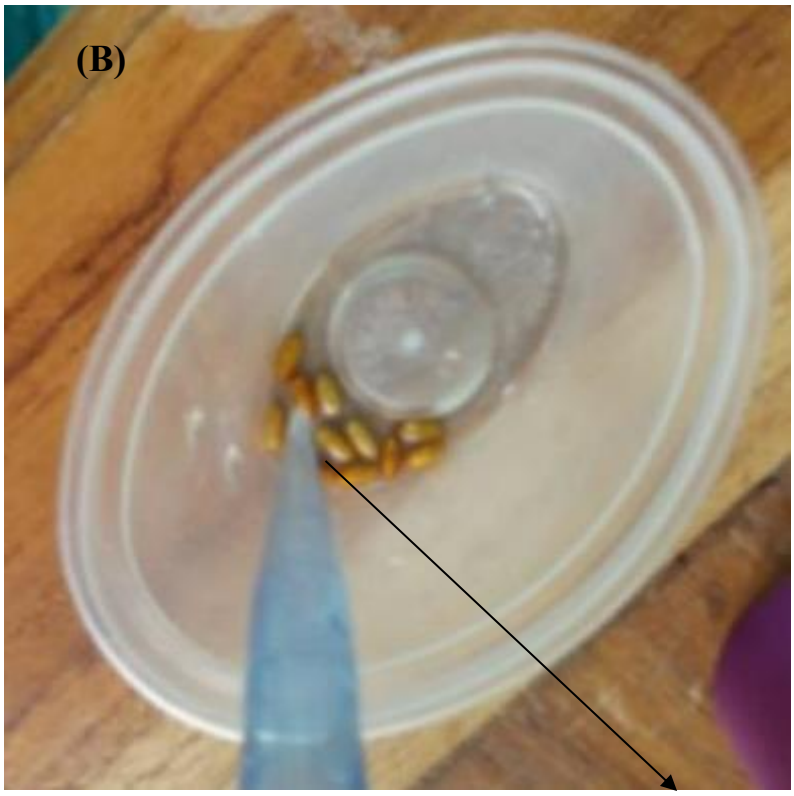
Host instar development (larvae, pupae and adults) were examined daily for dead and eventual fungal effect after inoculation. All dead host instars were removed from the boxes and placed on a sterile Whatman No 5 filter paper placed in a Petri dishes and humidified with Tween 80 (0.1%) solution to check for fungal growth for two weeks. Petri dishes were sealed with para-film and tagged with the correspondent date of death and number of cadavers. The number of enclosed pupae and /or adults from treated larvae and pupae was also recorded and incubated. Non-emerged pupae after 10 days post-inoculation was considered dead and the number recorded.

3.7.5 Effectiveness of *Beauveria bassiana* and *Metarhizium anisopliae* suspension on the fecundity of fruit fly species

A couple of ten adults (five females and five males) of *Z. cucurbitae* of 12 days old placed in plastic boxes of 3.5 x 4 cm and sprayed with different concentrations (10^5 to 10^{10} conidia/mL) of each conidia suspensions of entomopathogenic fungi. They were



Larvae of *Zeuzodacus cucurbitae*



Pupae of *Zeugodacus cucurbitae*

Plate 3.9. Inoculation of (A) larvae and (B) pupae of *Zeugodacus cucurbitae* with suspensions of entomopathogenic fungi

provided with the mixture of yeast and brown sugar. Punctured pieces of *Cucurbita pepo* var. magda-zucchini were placed inside each box for 48 hours. Control treatments were treated with sterile Tween 80 (0.1%) solution and another group was not treated but fed with the same diet. Five replications were done per concentration. Infested fruits were incubated for 10 days in a plastic box of 150 cm^3 and covered with muslin clothes maintained with an elastic band. The number of pupae collected from fruits exposed to infected adults and subsequent emerged adults were recorded.

3.7.6 Effect of local fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on *Zeugodacus cucurbitae* and *Dacus vertebratus*

This experiment was run in a Completely Randomized Design (CRD) with five replications per fruit fly species at 26 ± 1°C temperature, 70 ± 5% RH under a photoperiod of 12:12 (L: D). Ten third instar larvae of 5 to 7 days old of *D. vertebratus* and *Z. cucurbitae* placed separately in sterile plastic box of 3.5 x 4 cm was used per replicate. Conidial suspensions of isolated fungi (*Aspergillus niger, Fusarium verticillioides*, *Botryotricum* sp. and a bacterium, *Streptomycin* sp.) were individually mixed with suspensions of *B. bassiana* and *M. anisopliae* and was prepared for bioassay on mortality of *D. vertebratus* and *Z. cucurbitae*. The conidia suspensions made are presented in Table 3.3. Each conidia suspension was applied topically at a rate of 10^{10} conidial/mL onto the larvae. Control was sprayed with sterile Tween 80 (0.1%) solution. Inoculated larvae were incubated. Host instar development (larvae, pupae and adults) were daily examined for mortality and checked for fungal growth. Cadavers were monitored for two weeks for evidence of fungal growth mortality and mycosis of dead instars were monitored daily. The number of enclosed pupae and /or adults from treated larvae and pupae was noted.

3.8 Genotoxicity study of the fungal suspension using *Allium cepa* method

A genotoxicity of two most virulent isolates of *B. bassiana* was conducted through *Allium cepa* test. In this study, two selected isolates of *B. bassiana* (Bb337) and (Bb338) were tested for genotoxicity. The plant medium, onion (*Allium cepa*) was used. The purple colour variety of onion in average sized of 5 cm diameter were used. The plant media were dried under sun for 15 days.The dried roots at the base of the

Table 3.3. Conidia suspensions of indigenous fungi, *Beauveria bassiana* and *Metarhizium anisopliae* applied on *Zeugodacus cucurbitae* and *Dacus vertebratus*

Mixture of 50% of each isolate	Mixture of 50% of each isolate
50% Bb337 + 50% *A. niger*	100% Bb337 + 100% *A. niger*
50% Bb337 + 50% *F. verticillioides*	100% Bb337 + 100% *F. verticillioides*
50% Bb337 + 50% *Botryotricum* sp.	100% Bb337 + 100% *Botryotricum* sp.
50% Bb337 + 50% *Streptomycin* sp	100% Bb337 + 100% *Streptomycin* sp
50% Bb338 + 50% *A. niger*	100% Bb338 + 100% *A. niger*
50% Bb338 + 50% *F. verticillioides*	100% Bb338 + 100% *F. verticillioides*
50% Bb338 + 50% *Botryotricum* sp.	100% Bb338 + 100% *Botryotricum* sp.
50% Bb338 + 50% *Streptomycin* sp	100% Bb338 + 100% *Streptomycin* sp
50% MaD + 50% *A. niger*	100% MaD + 100% *A. niger*
50% MaD + 50% *F. verticillioides*	100% MaD + 100% *F. verticillioides*
50% MaD + 50% *Botryotricum* sp.	100% MaD + 100% *Botryotricum* sp.
50% MaD + 50% *Streptomycin* sp.	100% MaD + 100% *Streptomycin* sp.

onion bulbs were shaved off with a sharp knife to expose the fresh meristematic tissues. To determine the required suspensions of isolates, the surface occupied by a single mature watermelon was measured in the field. Concentration used to treat this surface was computed followed :

$$RC = (50g \times 4\pi r^2)/10000m^2$$

Where RC = Required Concentration and πr^2 = surface occupied by a single watermelon; 50 g = the quantity of entomopathgenic fungi required to treat 1ha of field surface (10000 m^2).

Suspensions of different isolates, Bb337 and Bb338 were prepared by dissolving 0.6 g of each isolate into a 50-mL graduated plastic. The conidial clumps were mixed with 20 mL sterilized distilled water of Tween 80 solution. The sterile conidia were vortexed/stirred for five minutes into a homogeneous suspension to dissociate conidial clumps. Five different concentrations of each conidia' suspensions were made (25%, 50%, 100%, 200% and 300%). Each concentration was replicated five times. Two control were made: sterilized 0.1 % Tween 80 solution and sterilized distilled water. They were also compared to the toxicity of the chemical insecticide, K-optimal. The base of the bulbs was placed on each prepared concentration mixed with distilled water in 150 ml test tubes (Plate 3.10). They were placed in the dark for 72 days. The roots of onions were measured and their morphological characters were observed.

3.9 Effectiveness of the most potent isolates of *Beauveria bassiana* in the control of fruit fly species infesting *Citrullus lanatus* under field conditions

This study was carried out to assess the potency of the two most virulent isolates of *B. bassiana* (Bb337 and Bb338) against *Z. cucurbitae* under laboratory conditions on fruit flies infesting *C. lanatus* in the field. They were compared with an isolate of *M. anisopliae* (MaD) from Dakar, a commercial bio-pesticide, *M. anisopliae* ICIPE 69 from Kenya and a chemical pesticide, K-optimal. Two experimental plots:, one was established in dry season and another in rainy season were set up. There was a control plots treated with sterile 0.1% Tween 80. A Randomized Complete Block Design with five replicates was used.

Plate 3.10. *Allium cepa* test design for genotoxicity study

3.9.1 Preliminary determination of time and frequency of application of different treatments

Seeds of watermelon were first sown on 2.4 m^2 plot spaced spaced out by 1 m width. Three plots were set for the preliminary test. The time of application was determined by applying suspensions of isolates three, four and five weeks after sowing. Suspensions were applied once, twice and three times per week to determine the frequency of application needed to efficiently reduce the population of fruit flies. Fruits were sampled before first application and 24 hours after each application. Data were collected on infestation rate by counting the number of pupae per fruit.

3.9.2 Field experimental set up for assessment of entomopathogenic against fruit flies on *Citrullus lanatus*

Field of watermelon was set up in the garden at IITA (Cotonou - Benin Republic). The experimental layout of about 35 m x 30 m was established and divided into 30 plots of 1.2 m x 2 m. Each plot was separated from the neighbouring plots by 2 m width. Similarly, each block was separated from neighbouring block by 2 m width (Figure 3.1). Seeds of watermelon were first sown on each plot. The experimental field was watered twice per day for the first four weeks and one time thereafter. Field was fertilized with poultry droppings at the rate of ½ kg per plants applied two weeks after sowing applied at 3 to 5 cm around a single plan. Plots were covered with mulching for moisture conservation (Plate 3.11). Two experimental sites were established during the dry and rainy seasons.

3.9.3 Field application of different treatment of entomopathogenic fungi

Five treatments including two isolates of *B. bassiana* (Bb337 and Bb338), two isolates of *M. anisopliae* (MaD and ICIPE-69) and a chemical pesticide K-optimal were applied in the experimental plots. Pre-spray samplings of fruits were made weekly until a week after the last treatment. Suspensions of *B. bassiana* (50 g / ha), *M. anisopliae* MaD (50 g / ha), ICIPE-69 (200 mL / ha) and K-optimal (1000 mL / ha) were applied. Different treatments were sprayed on the leaves and on the soil. Sprays were done in the afternoon (by 4 pm) three weeks after sowing and weekly for three weeks. Treatments were applied twice a week based on result from preliminary test on the determination of time and frequency of application of different treatments. Sampling was done a day before application and monitoring made daily, 24 h after each spray between 8 and 10 am.

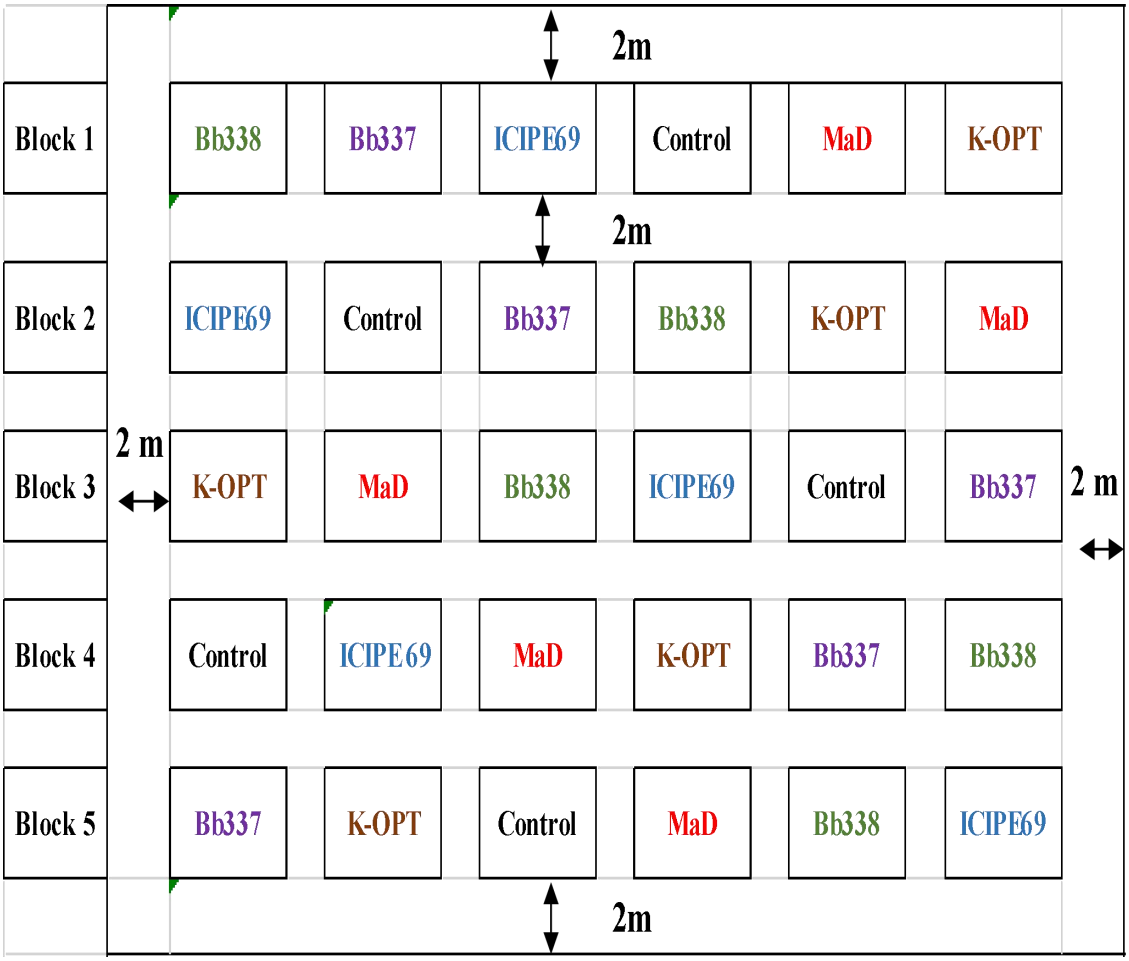


Figure 3.1. Randomized Complete Block Design for the assessment of entomopathogenic against fruit flies on *Citrullus lanatus*

Bb338 = *Beauvria bassiana* 338

BB337 = *Beauvria bassiana* 337

ICIPE69 = *Metarhizium anisopliae* ICIPE69

MaD = *Metarhizium anisopliae* Dakar

K-OPT = Chemical pesticide K-Optimal®

Plot size = 1.2m x 2m

3.9.3.1 Evaluation of the effect of fungal application on some beneficial organisms

Data were collected on the occurrence of beneficial insects whereby their presence was checked daily and recorded. The number of ants was assessed following standard procedures in terms of colony using a ranking category: 0 = 0; 1-5 = 1; 6-10 = 2; 11-15 = 3; >15 = 4. The infestation of fruit flies species was assessed through fruits sampling. Three fruits were randomly sampled from three selected plots per treatment the day before application. They were weighed and incubated at 26±2°C for 5 days. From each fruit, the number of pupae was recorded. Infestation rate was also determined through infested fruits collection and incubation. Fruits were weighed per plot and per treatment. Subsequent number of pupae produced was recorded. Infestation rate was derived from the formula described as follows (Cowley *et al.,* 1992):

$$IR = Np \div Wf$$

Where IR = Infestation rate, Np = Number of pupae and Wf = Weight of fruits.

Non-infested fruits were harvested two weeks after the last treatment and weighted per plot. Total yield per hectare (yha) was also computed:

$$yha = (WNIF \div SP)\, x\, 10000$$

Where WNIF = Weight of non-infested fruits, SP = Surface occupied by plants.

10000 = 1ha

3. 10 Statistical analysis

Data were analysed using descriptive statistics, ANOVA at α = 0.05 and different charts and tables were presented. Data following a multinomial distribution (percentage of adults capable of flying) were analysed using a Vector Generalized Linear Model (VGLM). Data following a Gamma distribution (larval, pupal and adult duration); a Zero Inflated Poisson distribution (abundance of spiders), gaussian distribution (sampling of infested fruits) were analysed using a Generalised Linear Model (GLM). GLM test was used to test the co-variables effect on the development time and longevity of fruit fly's species. Other data which violated the assumption for parametric tests (pupal weight, pupal dimension, and sex-ratio) were analysed with a Kruskal-Wallis Rank Sum test. Data on adult emergence rate were subjected to

Negative binomial (0.915). The negative binomial model was used for number of pupae. GLM, family binomial was used to analysed larval, pupae and adult mortality, and percent number of harvested fruits. Infestation by *D. vertebratus* was calculated as the ratio of number of adults per kilogram of fruit collected (infestation index) (Cowley *et al.,* 1992). It was suggested to Kruskal-Wallis Rank Sum test. Percent weight of harvested fruits was analysed using Kruskal test, p.adj = *"bonferroni"*). All the analyses were performed using R x 64 (version 3.2.5, R Development Core Team) statistical software.

CHAPTER FOUR

RESULTS

4.1 Occurrence, abundance and diversity of fungi with entomopathogenic potential associated with soils grown with vegetables crops in Benin Republic

The occurrence, abundance and diversity of fungi associated with soils where vegetables were grown under conventional practice significantly differed ($P < 0.05$) from organic farming practices in Benin Republic. A total of five fungi species including *Aspergillus niger*, *A. flavus*, *A. fumugatus*, *Botryotricum* sp. *Fusarium verticillioides* and an antibiotic, *Streptomycin* sp. were isolated using both insect bait and soil dilution methods (Plate 4.1). *Aspergillus fumugatus* was only isolated from soil used to grow *Citrullus lanatus* at Oganla Oganla with a number of 0.13 ± 0.09. Of the two methods used for the isolation of microorganisms, the serial dilution gave more concentration compare to insect bait. The number of *A. niger r*anged between 1.78 ±1.75 and 3.23 ± 2.08 with insect bait method and was significantly higher ($P < 0.05$) than others fungi species recorded. *Aspergilus flavus* was the lowest in number (0.00 ± 0.00 - 0.23 ± 0.59) in both isolation methods (Table 4.1).

The diversity and abundance of fungal species significantly differed from one locality to another ($P < 0.05$). *Aspergillus niger* had the highest number at Oganla (3.23 ± 2.08) as compared to Misserete (2.83 ± 2.07), Zinvie (2.42 ± 1.69), Ouando (2.28 ± 2.15), Agata (1.85 ± 1.84) and Akron (1.78 ± 1.74). Fungi were more abundant at Akron, Misserete and Zinvie where four different fungal species were encountered. Agata, Ouando and Oganla registered the lowest number of fungi species (Table 4.1)

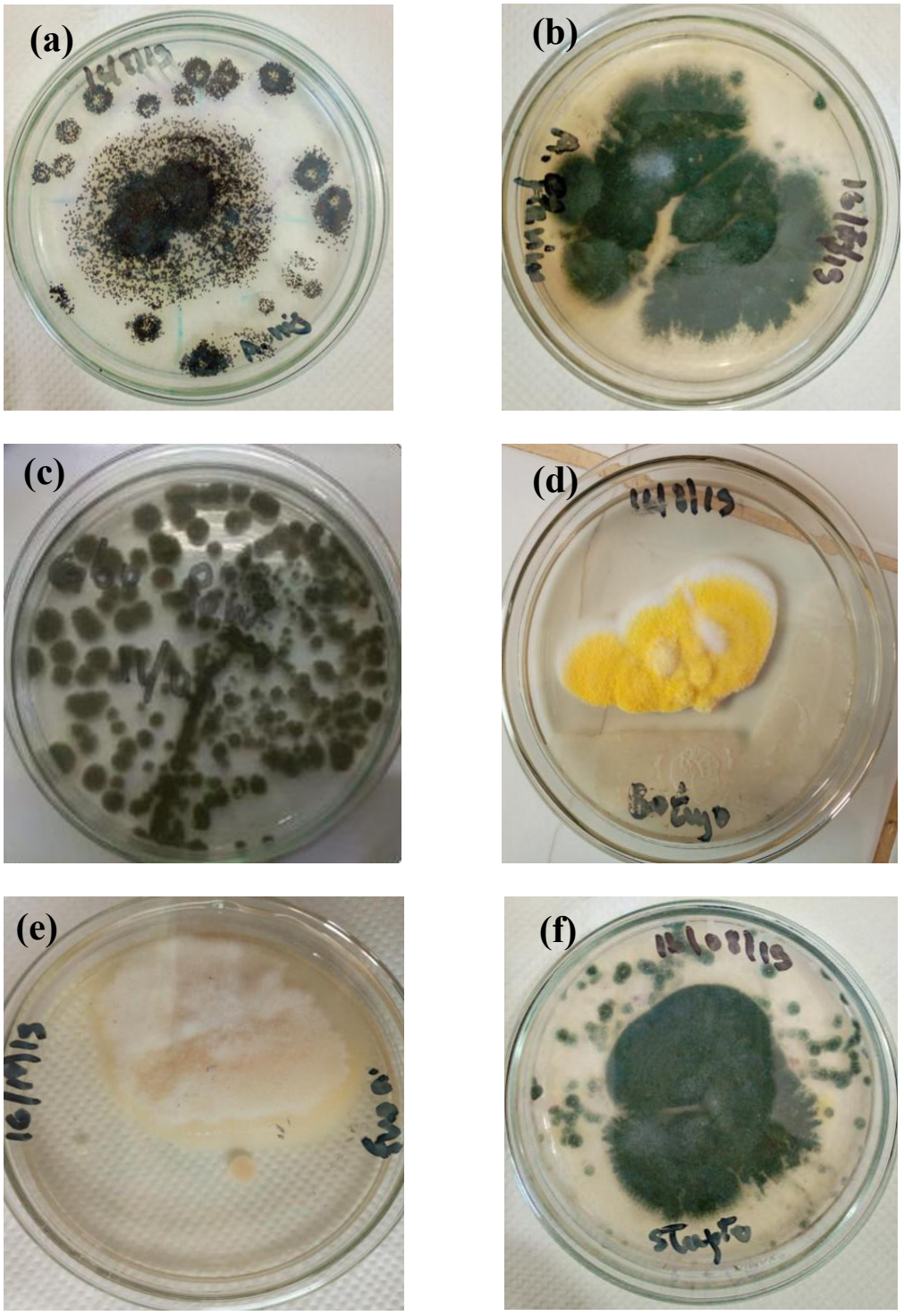


Plate 4.1. Fungi and bacterium species isolated from soil samples

a = Plate culture of *Aspergillus niger*
b = Plate culture of *Aspergillus flavus*
c = Plate culture of *Aspergillus fumugatus*
d = Plate culture of *Fusarium verticillioides*
e = Plate culture of *Botryotricum* sp.
f = Plate culture of *Streptomycin* sp.

Table 4.1. Number of fungi and bacterium species recorded in farming practices at different locality using Insect Bait and Soil Dilution Methods

Fungal isolation Methods	Fungi species	Conventional farming			Organic farming		
		Agata	Akron	Misserete	Oganla	Ouando	Zinvie
Soil Dilution Method	*Aspergillus flavus*	0.08 ± 0.28a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
	Aspergillus niger	0.39 ± 0.49b	0.39 ± 0.49b	0.39 ± 0.49b	0.53 ± 0.50ab	0.57 ± 0.50a	0.53 ± 0.50ab
	Botryotricum sp.	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	1.25 ± 0.33a
	F. verticilloides	0.00 ± 0.00b	0.07 ± 0.26a	0.11 ± 0.31a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
	Streptomycin sp.	0.06 ± 0.23b	0.24 ± 0.43a	0.24 ± 0.43a	0.26 ± 0.44a	0.18 ± 0.39ab	0.15 ± 0.36ab
Insect Bait Method	*Aspergillus flavus*	0.00 ± 0.00c	0.00 ± 0.00c	0.10 ± 0.30b	0.00 ± 0.00c	0.23 ± 0.59a	0.00 ± 0.00c
	Aspergillus niger	1.85 ± 1.84c	1.78 ± 1.74c	2.83 ± 2.07ab	3.23 ± 2.08a	2.28 ± 2.15bc	2.42 ± 1.69bc
	Botryotricum sp.	0.05 ± 0.22b	0.37 ± 0.96a	0.50 ± 0.97a	0.00 ± 0.00b	0.00 ± 0.00b	0.42 ± 1.08a
	F. verticilloides	0.00 ± 0.00b	0.35 ± 1.05a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.13 ± 0.47b
	Streptomycin sp.	0.50 ± 0.79c	0.43 ± 0.69c	1.10 ± 1.19b	0.77 ± 0.89bc	1.55 ± 1.84a	1.03 ± 1.29b

* Means within columns followed by the different letters are significantly different at $\alpha = 0.05$

4.1.1 Occurrence and abundance of fungi in soil used for growing vegetable crops in conventional and organic farming practices using insect bait method

The most abundant fungus in soil used for growing different vegetables crops using insect bait method was *Aspergillus niger* (0.95±0.24 to 4.37±0.61) in organic farming, significantly higher than (0.02±0.01 to 3.44±0.49) in conventional farming (Table 4.2 and 4.3). *Aspergillus niger* was significantly higher in soil used for growing *Citrullus lanatus* (3.44±0.49) than *Vernonia amygdalina* (3.31±0.48), *Amaranthus viridis* (3.00±0.46), *Cucumis sativus* (2.75±0.43) and *Capsicum annuum* (2.31±0.40). *Fusarium verticillioides* was not found in soil collected from Agata and Missérété. The number of *F. verticillioides* varied from soil used for growing *C. annuum* (0.31±0.09) to *A. viridis* (0.71±0.16) at Akron. Conversely, *A. flavus* was not obtained in soil samples collected from Akron and Agata. However, the number of *A. flavus* found at Missérété significantly varied from 0.11±0.05 (*A. viridis*) to 0.20±0.41 (*C. sativus*). *Botryotricum* sp. was more collected from soil used for growing *C. lanatus* (0.61±0.13) at Missérété and *A. viridis* (0.60±0.15) at Akron. The bacterium, *Streptomycin* sp. was significantly higher at Missérété as compared to Akron and Agata and varied from 0.85±0.17 (*Solanum lycopersicum*) to 1.29±0.22 (*V. amygdalina*). Fungi species were significantly different among vegetable crops from Agata and Akron ($P < 0.05$).

In organic farming, *A. niger* was more abundant in soil collected from all vegetable crops (0.95±0.24 to 4.37±0.61). The highest number of *A. niger* was obtained in soil used for growing *S. macrocarpon* (4.37±0.61) at Oganla. *Aspergillus flavus* were absent in soil samples collected from Zinvié. At Oganla, it was only found in soil used for growing *Amarantus viridis* (0.28±0.08). Conversely, *A. flavus* was found in soil samples obtained from Ouando with the highest number recorded on *Capsicum annuum* (0.37±0.11). Similarly, *Botryotricum* sp. and *F. verticillioides* were found in soil collected from Zinvié but not at Oganla. Both species were isolated from the same crop, *A. viridis* with the average density of 0.33±0.09 and 0.11±0.04 respectively at Ouando. The antibiotic, *Streptomycin* sp. was more abundant at Ouando. The number of isolated *Streptomycin* sp. ranged from 0.65 ± 0.17 on *L. sativa* to 2.44 ± 0.36 on *C. annuum.*

Table 4.2. Number fungi and bacterium species recorded from different vegetables crops and conventional farming practice on the occurrence and abundance of fungi using insect bait method

Location	Different vegetable Crops	*Aspergillus flavus*	*Aspergillus niger*	*Botryotricum* sp.	*Fusarium verticillioides*	*Streptomycin* sp.
Agata	*Amaranthus viridis*	0.00 ± 0.00c	1.85 ± 0.39a	0.05 ± 0.03c	0.00 ± 0.00c	0.50 ± 0.13b
	Capsicum annuum	0.00 ± 0.00b	0.02 ± 0.01b	0.00 ± 0.00b	0.00 ± 0.00b	0.21 ± 0.07a
	Cucumis sativus	0.00 ± 0.00c	2.08 ± 0.41a	0.06 ± 0.03c	0.00 ± 0.00c	0.56 ± 0.14b
	Solanum macrocarpon	0.00 ± 0.00c	2.24 ± 0.43a	0.06 ± 0.04a	0.00 ± 0.00a	0.60 ± 0.15b
	Solanum lycopersicum	0.00 ± 0.00b	0.08 ± 0.38b	0.05 ± 0.03b	0.00 ± 0.00b	0.48 ± 0.13a
	Vernonia amygdalina	0.00 ± 0.00c	2.39 ± 0.04a	0.06 ± 0.04c	0.00 ± 0.00c	0.65 ± 0.16b
Akron	*Amaranthus viridis*	0.00 ± 0.00c	2.92 ± 0.46a	0.60 ± 0.15b	0.57 ± 0.14b	0.71 ± 0.16b
	Cucumis sativus	0.00 ± 0.00c	2.01 ± 0.37a	0.41 ± 0.11b	0.39 ± 011b	0.49 ± 0.12b
	Ocimum gratissimum	0.00 ± 0.00c	2.19 ±0.39a	0.45 ± 0.12b	0.43 ± 0.11b	0.53 ± 0.13b
	Solanum macrocarpon	0.00 ± 0.00c	1.70 ± 0.34a	0.35 ± 0.10b	0.33 ± 0.09b	0.41 ± 0.11b
	Capsicum annuum	0.00 ± 0.00b	0.61 ± 0.20a	0.13 ± 0.05b	0.12 ± 0.04b	0.14 ± 0.05b
	Vernonia amygdalina	0.00 ± 0.00c	1.27 ± 0.08a	0.26 ± 0.08b	0.25 ± 0.07b	0.31 ± 0.09b
Missérété	*Amaranthus viridis*	0.11 ± 0.05c	3.00 ± 0.46a	0.53 ± 0.12b	0.00 ± 0.00a	1.16 ± 0.21b
	Capsicum annuum	0.08 ± 0.06c	2.31 ± 0.40a	0.41 ± 0.10bc	0.00 ± 0.00c	0.90 ± 0.18b
	Citrullus lanatus	0.12 ± 0.05d	3.44 ± 0.49a	0.61 ± 0.13c	0.00 ± 0.00d	1.33 ± 0.23b
	Cucumis sativus	0.20 ± 0.41c	2.75 ± 0.43a	0.49 ± 0.11bc	0.00 ± 0.00c	1.07 ± 0.20b
	Solanum lycopersicum	0.08 ± 0.03c	2.19 ± 0.38a	0.39 ± 0.09c	0.00 ± 0.00c	0.85 ± 0.17b
	Vernonia amygdalina	0.12 ± 0.05c	3.31 ± 0.48a	0.58 ± 0.13b	0.00 ± 0.00c	1.29 ± 0.22b

* Means within rows followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.3. Number of fungi and bacterium species recorded among vegetables crops from selected organic farming practice using Insect Bait Method

Locality	Different vegetable Crops	*Aspergillus flavus*	*Aspergillus niger*	*Botryotricum sp.*	*Fusarium verticillioides*	*Streptomycin* sp.
Oganla	*Amaranthus viridis*	0.00 ± 0.00c	3.31 ± 0.38a	0.00 ± 0.00c	0.00 ± 0.00c	0.79 ± 0.14b
	Capsicum annuum	0.00 ± 0.00c	2.43 ± 0.45a	0.00 ± 0.00c	0.00 ± 0.00c	0.58 ± 0.13b
	Citrullus lanatus	0.00 ± 0.00c	3.48 ± 0.54a	0.00 ± 0.00c	0.00 ± 0.00c	0.82 ± 0.17b
	Cucumis sativus	0.00 ± 0.00c	2.51 ± 0.46a	0.00 ± 0.00c	0.00 ± 0.00c	0.59 ± 0.13b
	Solanum macrocarpon	0.00 ± 0.00c	4.37 ± 0.61a	0.00 ± 0.00c	0.00 ± 0.00c	1.04 ± 0.20b
	Amaranthus viridis	0.28 ± 0.08c	2.75 ± 0.42a	0.00 ± 0.00c	0.00 ± 0.00c	1.87 ± 0.31b
Ouando	*Capsicum annuum*	0.37 ± 0.11c	3.59 ± 0.50a	0.00 ± 0.00c	0.00 ± 0.00c	2.44 ± 0.36b
	Daucus carota	0.14 ± 0.05c	1.40 ± 0.29a	0.00 ± 0.00c	0.00 ± 0.00c	0.96 ± 0.21b
	Lactuva sativa	0.20 ± 0.24b	0.95 ± 0.24a	0.00 ± 0.00b	0.00 ± 0.00b	0.65 ± 0.17a
	Capsicum pepper	0.24 ± 0.07c	2.30 ± 0.38a	0.00 ± 0.00c	0.00 ± 0.00c	1.56 ± 0.28b
	Solanum macrocarpon	0.28 ± 0.08c	2.70 ± 0.42a	0.00 ± 0.00c	0.00 ± 0.00c	1.83 ± 0.30b
	Amarantus viridis	0.00 ± 0.00c	1.93 ± 0.36a	0.33 ± 0.09c	0.11 ± 0.04c	0.83 ± 0.17b
Zinvié	*Celosia sp.*	0.00 ± 0.00c	1.87 ± 0.35a	0.31 ± 0.08c	0.10 ± 0.04c	0.80 ± 0.17b
	Lactuva sativa	0.00 ± 0.00d	4.29 ± 0.56a	0.74 ± 0.17c	0.24 ± 0.09d	1.83 ± 0.30b
	Petroselium crispum	0.00 ± 0.00c	2.18 ± 0.38a	0.38 ± 0.10c	0.12 ± 0.05c	0.93 ± 0.16b
	Solanum nigrum	0.00 ± 0.00c	1.33 ± 0.29a	0.23 ± 0.07c	0.07 ± 0.07c	0.57 ± 0.14bc
	Vernonia amygdalina	0.00 ± 0.00d	2.90 ± 0.45a	0.50 ± 0.12c	0.16 ± 0.06d	1.24 ± 0.22b
	Amarantrus viridis	0.00 ± 0.00c	3.31 ± 0.38a	0.00 ± 0.00c	0.00 ± 0.00c	0.79 ± 0.14b

* Mean within rows followed by the same letters are not significantly different at = 0.05

4.1.2 Occurrence and abundance of fungi species in soil used for growing different vegetable crops in conventional and organic farming practices using soil dilution

The occurrence and abundance of fungi species were not significant ($P > 0.05$) among soil dilutions (10^{-3} to 10^{-5}). *Aspergillus niger* and the bacterium *Streptomycin* sp. were the most abundant microorganism isolated from soil samples. The number of *A. niger* significantly ($P < 0,05$) varied from 0.25 ± 0.10 (*Cucumis sativus*) to 0.50 ± 0.11 (*Amaranthus viridis*) at Akon; from 0.07 ± 0.04 (*Amaranthus viridis*) to 0.58 ± 0.11 (*C. sativus*) at Agata; and from 0.28 ± 0.10 (*Solanum lycopersicum*) to 0.47 ± 0.11 (*V. amygdalina*, *A. viridis* and *Capsicum annuum*) at Misserete. The antibiotic, *Streptomycin* sp. was less abundant at Agata (0.02 ± 0.02 from *A. viridis* to 0.10 ± 0.06 from *C. sativus*) as compared to samples from Akron (*C. sativus* = 0.13 ± 0.06 - *A. viridis* = 0.32 ± 0.10) and Misserete (*S. lycopersicum* = 0.15 ± 0.07 – *C. annuum* 0.30 ± 0.10). *Botryotricum* sp. was not found in any of the three selected conventional farming practice. *Aspergillus flavus* was present at Agata only and its number is higher in soil used for growing *Amaranthus viridis* (0.38 ± 0.12) as compared to *Cucumis sativus* (0.15 ± 0.07), *Solanum macrocarpon* (0.11 ± 0.06), *S. lycopersicum* (0.07 ± 0.04), *V. amygdalina* (0.06 ± 0.04) and *Capsicum annum* (0.03 ± 0.02). *F. verticillioides* was present at both Akron and Missérété. However, no significant differences were noted among fungi species isolates from different soil samples collected from crops obtained at Agata and Akron (Table 4.4).

In organic farming practice, *A. flavus* and *F. verticillioides* were not found. *Bostryotricum* sp. was only collected from soil collected at Zinvié and significantly varied from 0.04 ± 0.03 *Petroselium crispum*) to 0.39 ± 0.12 (*Celosia* sp.). The number of *A. niger* was significantly higher at Zinvié and ranged from 0.33 ± 0.12 (*P. crispum*) to 0.84 ± 0.07 (*S. nigrum*) as compared to Ouando (*Capsicum pepper* = 0.42 ± 0.13 - *Lactuva sativa* = 0.74 ± 0.10) and Oganla (*Solanum macocarpon* = 0.36 ± 0.12 – *A. viridis* = 0.64 ± 0.08). The antibiotic, *Streptomycin* sp. was comparatively more abundant at Oganla (*S. macrocarpon* = 0.14 ± 0.07 – *A. viridis* = 0.36 ± 0.08) than at Zinvie (*P. crispum* = 0.05 ± 0.03 - *S. nigrum* = 0.36 ± 0.01) and Ouando (*C. pepper* = 0.08 ± 0.05 - *Lactuva sativa* = 026 ± 0.10). Fungi species were significantly ($P < 0.05$) different among crops from Zinvié (Table 4.5).

Table 4.4. Number of fungi and bacterium species recorded among vegetables crops from three selected conventional farming practice using soil dilution method

Localities	Different vegetable Crops	*Aspergillus flavus*	*Aspergillus niger*	*Botryotricum sp.*	*Fusarium verticillioides*	*Streptomycin* sp.
Agata	*Amaranthus viridis*	0.38 ± 0.12a	0.07 ± 0.04b	0.00 ± 0.00b	0.00 ± 0.00b	0.05 ± 0.03b
	Capsicum annuum	0.03 ± 0.02b	0.20 ± 0.10a	0.00 ± 0.00b	0.00 ± 0.00a	0.02 ± 0.02a
	Cucumis sativus	0.15 ± 0.07b	0.58 ± 0.11a	0.00 ± 0.00b	0.00 ± 0.00b	0.10 ± 0.06b
	Solanum macrocarpon	0.11 ± 0.06b	0.48 ± 0.12a	0.00 ± 0.00b	0.00 ± 0.00b	0.07 ± 0.04b
	Solanum lycopersicum	0.07 ± 0.04b	0.38 ± 0.12a	0.00 ± 0.00b	0.00 ± 0.00b	0.05 ± 0.03b
	Vernonia amygdalina	0.06 ± 0.04b	0.32 ± 0.12a	0.00 ± 0.00b	0.00 ± 0.00b	0.04 ± 0.03b
Akron	*Amaranthus viridis*	0.00 ± 0.00b	0.50 ± 0.11a	0.00 ± 0.00b	0.10 ± 0.05b	0.32 ± 0.10a
	Cucumis sativus	0.00 ± 0.00b	0.25 ± 0.10a	0.00 ± 0.00b	0.04 ± 0.02b	0.13 ± 0.06b
	Ocimum gratissimum	0.00 ± 0.00b	0.42 ± 0.11a	0.00 ± 0.00b	0.08 ± 0.04b	0.26 ± 0.09b
	Solanum macrocarpon	0.00 ± 0.00b	0.38 ± 0.11a	0.00 ± 0.00b	0.06 ± 0.04b	0.22 ± 0.08b
	Capsicum annuum	0.00 ± 0.00b	0.42 ± 0.11a	0.00 ± 0.00b	0.08 ± 0.04b	0.26 ± 0.09b
	Vernonia amygdalina	0.00 ± 0.00b	0.38 ± 0.11a	0.00 ± 0.00b	0.06 ± 0.04b	0.22 ± 0.08b
Missérété	*Amaranthus viridis*	0.00 ± 0.00b	0.47 ± 0.11a	0.00 ± 0.00b	0.15 ± 0.06b	0.30 ± 0.10a
	Capsicum annuum	0.00 ± 0.00b	0.47 ± 0.11a	0.00 ± 0.00b	0.15 ± 0.06b	0.30 ± 0.10a
	Citrullus lanatus	0.00 ± 0.00b	0.32 ± 0.10a	0.00 ± 0.00b	0.08 ± 0.04b	0.18 ± 0.07b
	Cucumis sativus	0.00 ± 0.00b	0.32 ± 0.10a	0.00 ± 0.00b	0.08 ± 0.04b	0.18 ± 0.07b
	Solanum lycopersicum	0.00 ± 0.00b	0.28 ± 0.10a	0.00 ± 0.00b	0.07 ± 0.04b	0.15 ± 0.07b
	Vernonia amygdalina	0.00 ± 0.00b	0.47 ± 0.11a	0.00 ± 0.00b	0.15 ± 0.06b	0.30 ± 0.09a

* Mean within rows followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.5. Number of fungi and bacterium species recorded among vegetables crops from three selected organic farming practice using soil dilution method

Locality	Crops	*Aspergillus flavus*	*Aspergillus niger*	*Botryotricum* sp.	*Fusarium verticillioides*	*Streptomycin* sp.
Oganla	*Amarantus viridis*	0.00 ± 0.00c	0.64 ± 0.08a	0.00 ± 0.00c	0.00 ± 0.00c	0.36 ± 0.08b
	Capsicum annuum	0.00 ± 0.00b	0.41 ± 0.12a	0.00 ± 0.00b	0.00 ± 0.00b	0.17 ± 0.07b
	Citrullus lanatus	0.00 ± 0.00c	0.56 ± 0.11a	0.00 ± 0.00c	0.00 ± 0.00c	0.28 ± 0.10b
	Cucumis sativus	0.00 ± 0.00c	0.56 ± 0.11a	0.00 ± 0.00c	0.00 ± 0.00c	0.28 ± 0.10b
	Solanum macocarpon	0.00 ± 0.00b	0.36 ± 0.12a	0.00 ± 0.00b	0.00 ± 0.00b	0.14 ± 0.07b
Ouando	*Capsicum annuum*	0.00 ± 0.00b	0.65 ± 0.11a	0.00 ± 0.00b	0.00 ± 0.00b	0.18 ± 0.08b
	Daucus carota	0.00 ± 0.00b	0.54 ± 0.12a	0.00 ± 0.00b	0.00 ± 0.00b	0.13 ± 0.06b
	Lactuva sativa	0.00 ± 0.00b	0.74 ± 0.10a	0.00 ± 0.00b	0.00 ± 0.00b	0.26 ± 0.10b
	Capsicum pepper	0.00 ± 0.00b	0.42 ± 0.13a	0.00 ± 0.00b	0.00 ± 0.00b	0.08 ± 0.05b
	Solanum macocarpon	0.00 ± 0.00b	0.48 ± 0.13a	0.00 ± 0.00b	0.00 ± 0.00b	0.10 ± 0.05b
Zinvié	*Amarantus viridis*	0.00 ± 0.00c	0.79 ± 0.08a	0.24 ± 0.09b	0.00 ± 0.00c	0.30 ± 0.10b
	Celosia sp.	0.00 ± 0.00b	0.39 ± 0.12a	0.39 ± 0.12b	0.00 ± 0.00b	0.06 ± 0.04b
	Lactuva sativa	0.00 ± 0.00b	0.39 ± 0.12a	0.05 ± 0.03b	0.00 ± 0.00b	0.06 ± 0.04b
	Petroselium crispum	0.00 ± 0.00b	0.33 ± 0.12a	0.04 ± 0.03b	0.00 ± 0.00b	0.05 ± 0.03b
	Solanum nigrum	0.00 ± 0.00c	0.84 ± 0.07a	0.30 ± 0.10b	0.00 ± 0.00c	0.36 ± 0.01b
	Vernonia amygdalina	0.00 ± 0.00b	0.44 ± 0.12a	0.06 ± 0.04b	0.00 ± 0.00b	0.08 ± 0.04b

* Mean within rows followed by the same letters are not significantly different at $\alpha = 0.05$

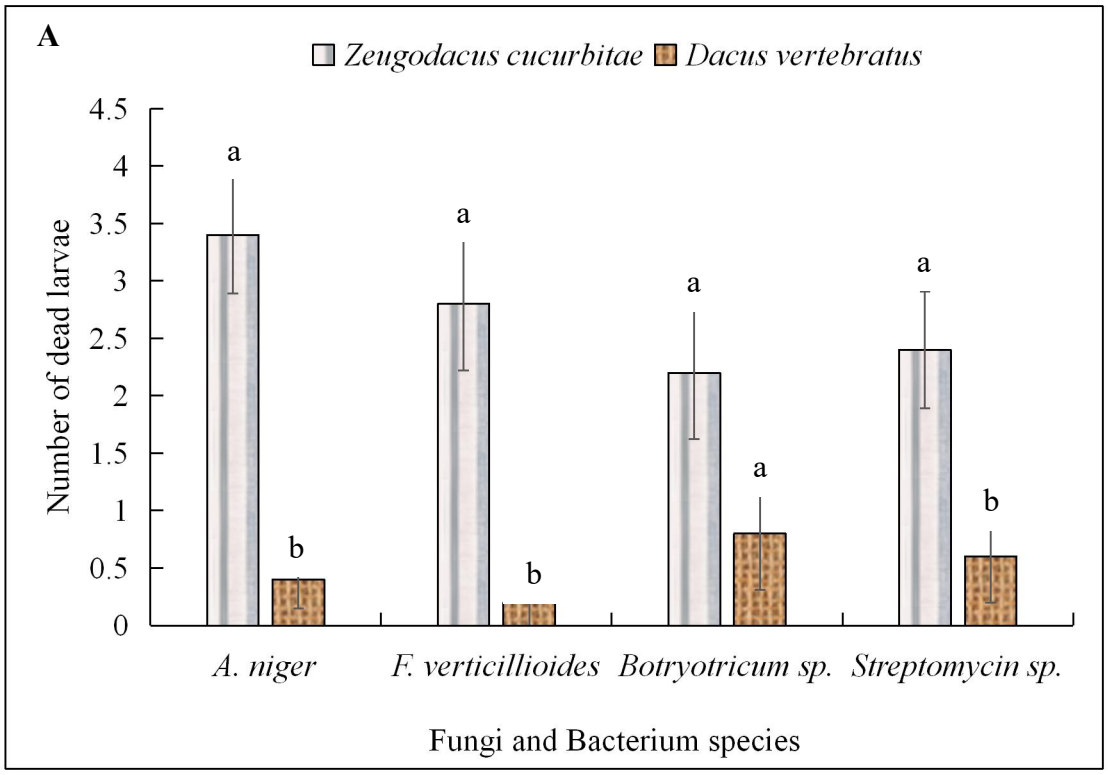
4.1.3 Effect of isolated fungi with entomopathogenic potential on larvae and enclosed pupae of *Dacus vertebratus* and *Zeugodacus cucurbitae*

The entomapathogenic potential of each isolated fungi and bacterium species on larvae of two fruit fly species, *D. vertebratus* and *Z. cucurbitae* is presented below.

4.1.3.1 Efficacy of different fungi and bacteria species on larvae mortality of *Dacus vertebatus* and *Zeugodacus cucurbitae*

Efficacy of newly isolated fungi from soil samples (*Aspergillus niger*, *Fusarium verticillioides* and *Botriotrycum* sp.) and an antibiotic, *Streptomycin* sp. evaluated on the larvae of *D. vertebatus* and *Z. cucurbitae* is shown in Figure 4.1a. The number of dead larvae of *Z. cucurbitae* was significantly higher after inoculation with all fungi and bacterium species than mortality recorded with *D. vertebratus* ($P < 0.05$). *Aspergillus niger* significantly affected larvae of *Z. cucurbitae* causing a higher mean larval mortality (3.40 ± 0.51) as compared to *D. vertebatus* (0.40 ± 0.25). Similarly, *F. verticillioides* and the antibiotic, *Streptomycin* sp. significantly induced high *Z. Cucurbita* larval mortality of 2.80 ± 0.58 and 2.4 ± 0.51 which was greater than *D. vertebratus* larval mortality of 0.20 ± 0.20 and 0.60 ± 0.40. Conversely, larval mortality caused by *Botryotricum* sp. to *Z. Cucurbita* (2.20 ± 0.49) was significantly different from that of *D. vertebratus* (0.60 ± 0.40) ($P < 0.05$).

The effectiveness of different fungi and bacterium species confirmed by fungal growth on cadavers' larvae in the presence of mycosis is shown in Figure 4.1b. The highest number of cadavers' larvae with mycosis was recorded on cadavers larvae of *Z. cucurbitae* inoculated with the antibotic, *Streptomycin* sp. (0.60 ± 0.24). However, no bacterial growth was observed on cadavers of *D. vertebratus* larvae when inoculated with *Streptomycin* sp. Similarly, cadavers of *Z. cucurbitae* larvae showed no fungal growth after treatment with *F. verticillioides* while, mycosis was observed on cadavers of *D. vertebratus* larvae (0.20 ± 0.20). Conversely, mycosis of *A. niger* was found on both cadavers of *D. vertebratus* (0.40 ± 0.24) and *Z. cucurbitae* larvae (0.20 ± 0.20). However, mean number of cadaver larvae with mycosis of *A. niger* was not significantly different among fruit fly species ($P > 0.05$).



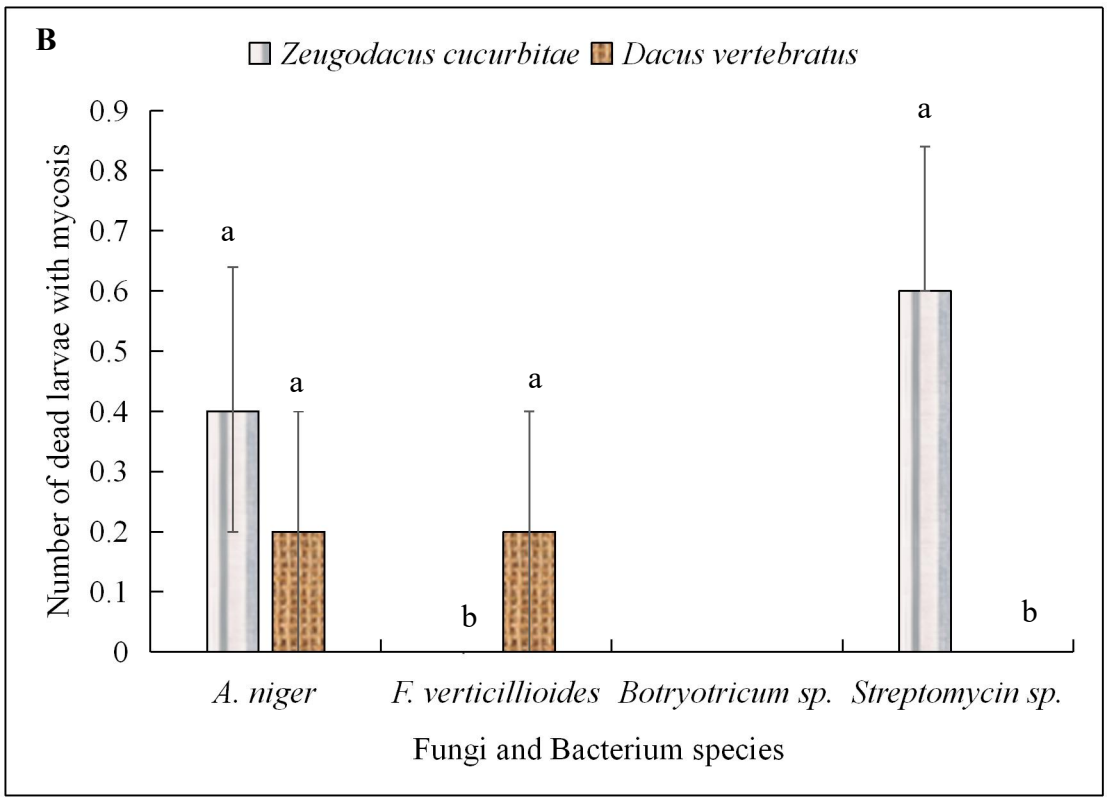


Figure 4.1. Number of A) dead larvae after inoculation with different fungi and bacterium species and B) cadavers of *Zeugodacus cucurbitae* and *Dacus vertebratus* larvae with fungal growth

* Bars with same letter are different among fruit fly species at α=0.05, Wilcoxon rank test

4.1.3.2 Efficacy of different fungi and bacteria species on enclosed pupae of *Dacus vertebatus* and *Zeugodacus cucurbitae*

Efficacy of different fungi and bacterium species on enclosed pupae of both fruit fly species, *D. vertebratus* and *Z. cucurbitae* is shown in Figure 4.2a. Pupae formed (enclosed pupae) after larval inoculation by fungi and bacterium species confirmed the vertical transmission of fungal spores from one stage to another. All fungi and bacterium species caused death in enclosed pupae with no significant difference among fruit fly species. The highest mean number of dead pupae was recorded on *Z. cucurbitae* (3.80 ± 0.58) which was not significantly higher than *D. vertebratus* (2.60 ± 0.51) with *Botryotricum* sp. used as treatment. Similarly, *A. nige*r and the antibiotic, *Streptomycin* sp. caused high pupae mortality in *Z. cucurbitae* (3.40 ± 0.24) and (2.20 ± 0.58) as compared to *D. vertebratus* (2.80 ± 0.37) and (1.60 ± 0.24) respectively. In contrast, the highest number of dead pupae was recorded on *D. vertebratus* (3.20 ± 0.58) with the lowest on *Z. cucurbitae* (3.00 ± 0.55) when inoculated with *F. verticillioides.*

The evidence of fungal efficacy was confirmed by fungal growth on enclosed pupae cadavers (presence of mycosis). Figure 4.2b showed the mean number of pupae cadavers of *D. vertebratus* and *Z. cucurbitae* with mycosis. Significant difference was not observed in number of cadavers of both fruit fly species and among fungi and bacterium species. Mycosis was not observed on cadaver of *D. vertebratus* pupae while from *Z. cucurbitae* pupae cadavers, there was fungal growth (0.20 ± 0.20). The number of cadavers of *Z. cucurbitae* pupae was high (0.60 ± 0.40) as compared to *D. vertebratus* (0.20 ± 0.20) when *F. verticillioides* was used as treatment. Conversely, high number of pupae cadavers with *Botryotricum* sp. mycosis was recorded on *D. vertebratus* (0.40 ± 0.24) with the lowest on *Z. cucurbitae* (0.20 ± 0.20). Same number of cadavers with the antibiotic, *Streptomycin* sp. was recorded for both fruit fly species (0.20 ± 0.20).

4.1.3.3 Efficacy of different fungi and bacteria species on enclosed adult emergence of *Dacus vertebatus* and *Zeugodacus cucurbitae*

Number of emerged flies from enclosed pupae after larvae inoculation is shown in Figure 4.3a. Significant difference was not observed among number of emerged *D. vertebratus* and *Z. cucurbitae* for each of fungi and bacterium species. The highest number of emerged flies was recorded on *D. vertebratus*.

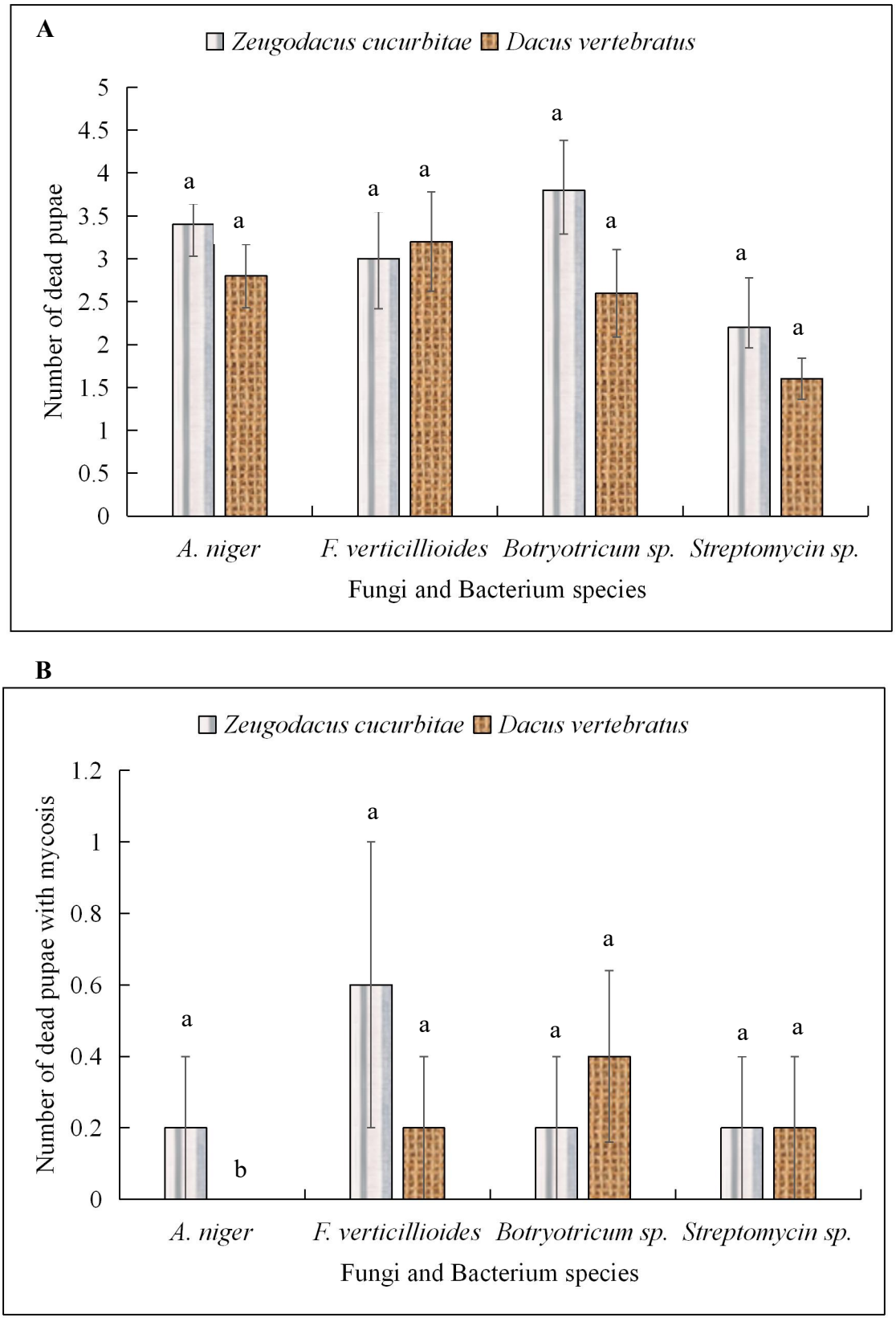


Figure 4.2. Number of A) dead pupae of after inoculation with different fungi and bacterium species and B) cadavers of *Zeugodacus cucurbitae* and *Dacus vertebratus* pupae with fungal growth

* Bars with same letter are different among fruit fly species at α=0.05, Wilcoxon rank test

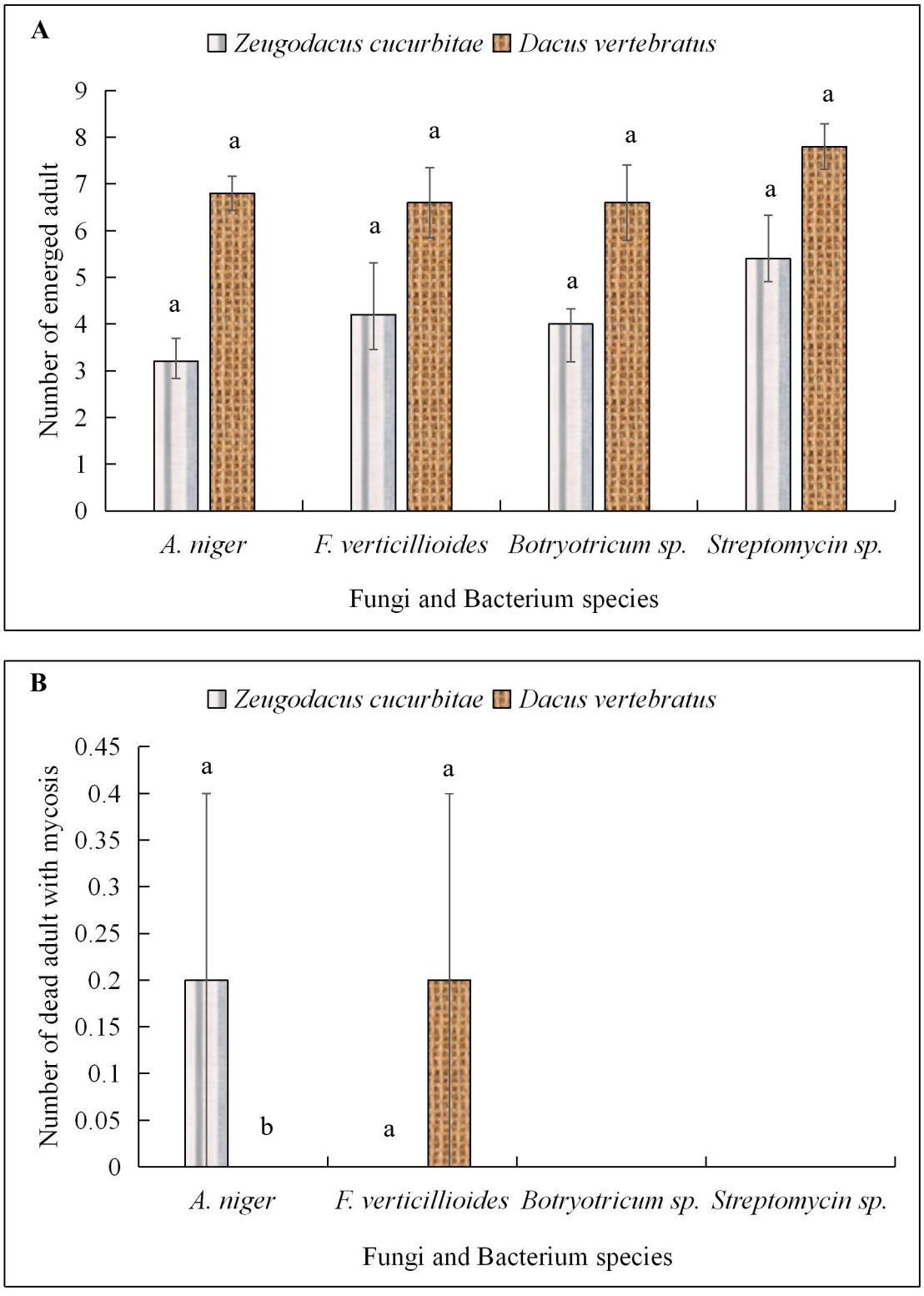


Figure 4.3. Number of A) emerged adult after inoculation with different fungi and bacterium species and B) cadavers adult with fungal growth

* Bars with same letter are different among fruit fly species at α=0.05, Wilcoxon rank test

The antibiotic, *Streptomycin* sp. favoured more *D. vertebratu*s adult emergence (7.80 ± 0.49) higher than the number of emerged *Z. cucurbitae* (5.40 ± 0.93). Similarly, *A. niger*, *F. verticillioides* and *Botryotricum* sp. encountered number of emerged *D. vertebratus* (6.80 ± 0.37), (6.60 ±0.75) and (6.60 ± 0.81) higher than *Z. cucurbitae* (3.20 ± 0.49), (4.40 ± 1.11) and (4.00 ± 0.32) respectively.

Figure 4.3b shows dead adult flies of *D. vertebratus* and Z. *cucurbitae* with fungal growth (mycosis) that emerged from enclosed pupae after larvae inoculation Cadavers of adult flies with fungal and bacterial growth were not significantly different among fungi and bacterium species. Also, no significant difference was noted in the number of adult cadavers showing fungal among fruit fly species. Cadavers of both *D. vertebratus* and *Z. cucurbitae* adults shown no fungal and bacterial growth after treatment with *Botryotricum* sp. No mycosis was recorded on adult cadavers of *Z. cucurbitae* when inoculation with *F. verticillioides.* Similarly, adult cadavers of *D. vertebratus* shown no fungal growth with *A. niger* used as treatment. However, fungal growth was observed on adult cadavers of *Z. cucurbitae* (0.20 ± 0.20) and *D. vertebratus* (0.20 ± 0.20) with *A. niger* and *F. verticillioides* respectively.

4.2 Life cycle of *Dacus vertebratus* and *Zeugodacus cucurbitae* infesting *Citrullus lanatus*

The life cycle of *D. vertebratus* and *Z. Cucurbita*are presented in terms of duration of each instar development (days), morphometrics (mm) and survival (%) of immature stages on watermelon.

4.2.1 Description and morphometric measurements of immature stages of *Zeugodacus cucurbitae* on *Citrullus lanatus*

The two fruit flies, *D. vertebratus* and *Z. cucurbitae* are holometabolous with four stages: egg, larva, pupa and adult in their development and had three larval instars (Plate 4.2). Morphometric measurements of each instar of development are presented in Table 4.6. The freshly laid eggs of *Z. cucurbitae* were white in colour, cylindrical, elongated and slightly curved and measured in average 1.05 ± 0.02 mm in length and 0.17 ± 0.00 mm in width. Eggs were laid in batches of 3 to 12 eggs at about mm in the epidermis of the fruit. First instar larvae were white and cream with a mean length of 2.61 ± 0.00 mm and 0.51 ± 0.04 mm in width. Second instar was whitish to slightly

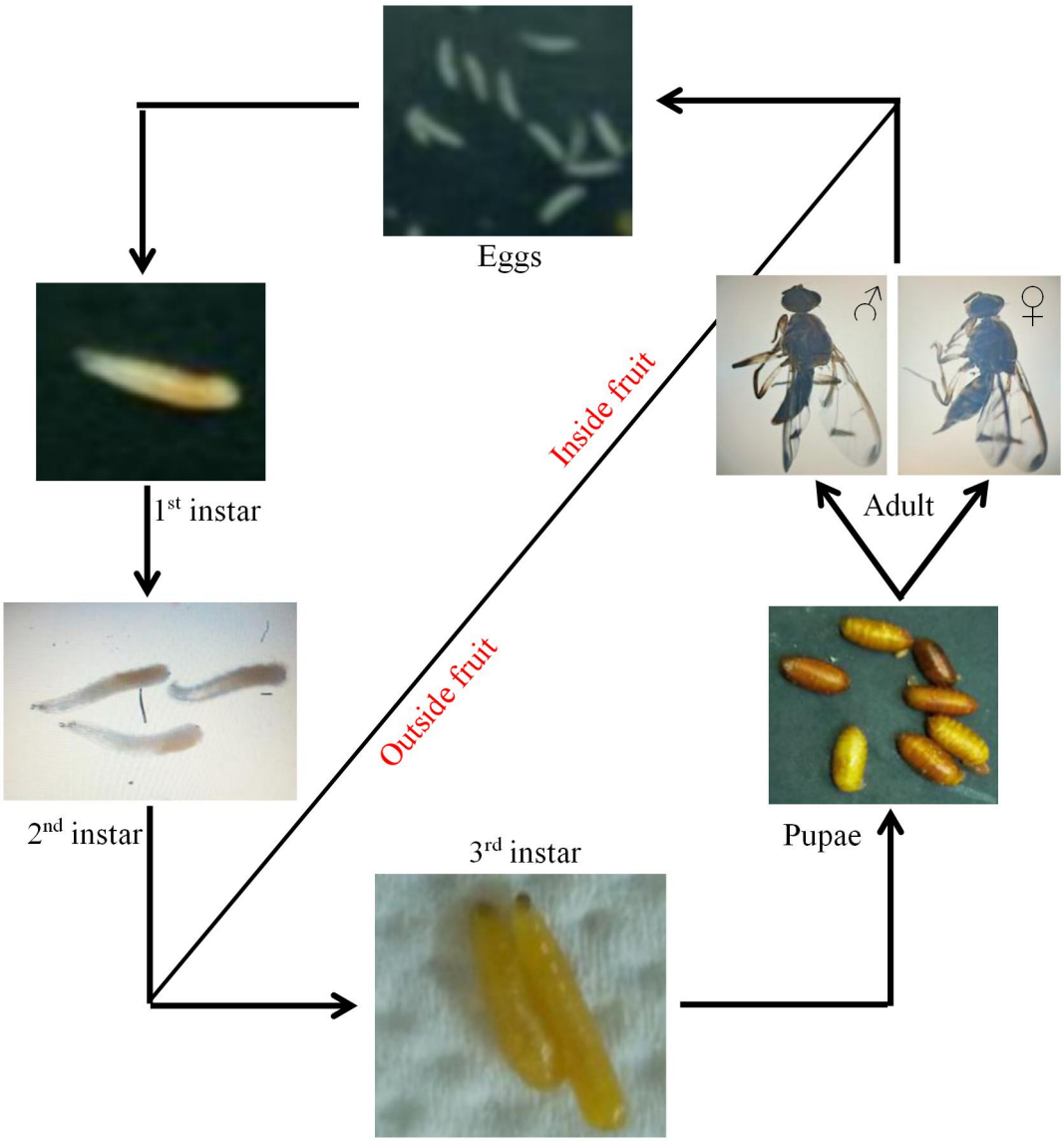


Plate 4.2. Life cycle of *Zeugodacus cucurbitae*

Magnification : x 200

Table 4.6. Body length and width measurements of *Zeugodacus cucurbitae* and *Dacus vertebratus*

	Body length measurements (mm)						
Fruit fly species	Eggs	First instar	Second instar	Third instar	Pupae	Adult female	Adult male
Zeugodacus cucurbitae	1.05 ± 0.02a	2.61 ± 0.08a	4.43 ± 0.65a	7.78 ± 0.16a	5.19 ± 0.17a	6.36 ± 0.30a	8.23 ± 0.17b
Dacus vertebratus	1.02 ± 0.02a	2.74 ± 0.07a	4.58 ± 0.14a	7.60 ± 0.14a	5.22 ± 0.16a	6.26 ± 0.27a	6.33 ± 0.18a
	Body width measurements (mm)						
Zeugodacus cucurbitae	0.17 ± 0.00a	0.51 ± 0.04a	0.74 ± 0.02b	1.19 ± 0.02a	1.92 ± 0.09a	2.14 ± 0.14a	2.39 ± 0.12a
Dacus vertebratus	0.18 ± 0.00a	0.49 ± 0.04a	0.67 ± 0.02a	1.18 ± 0.02a	1.97 ± 0.07a	2.08 ± 0.11a	2.16 ± 0.10a

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

yellowish measuring 4.43 ± 0.65 mm in length and 0.74 ± 0.02 mm in width. The third instar which jump into the soil for pupation was however yellowish in colour. The length of third instar of *Z. vertebratus* was 7.78 ± 0.16 mm and 1.19 ± 0.02 mm in width. The newly formed pupae were light brown before turning slightly to brown colour. Pupae were cylindrical shaped and measured 5.19 ± 0.17 mm in length and 1.92 ± 0.09 mm in width. Adult of *Z. cucurbitae* was yellowish to brown in colour having three yellow lateral and median marking (stries) on the scutum situated on the thorax. On the wing, brown patches were noted and black bands on the radio-medial veins were observed. The R-costal of the wing was well developed with an enlarged apical spot. The pre-apical had a transversal band. The abdomen was characterized by the presence of T-like band on the 3-5 tergit. Females were of 6.36 ± 0.30 mm and 2.14 ± 0.14 mm smaller than males measuring 8.23 ± 0.17 mm and 2.39 ± 0.12 mm in length and width respectively. Females were different from the female by the presence of tapering abdomen extending into an ovipositor.

4.2.2 Description and morphometric measurements of immature stages of *Dacus vertebratus* on *Citrullus lanatus*

The fruit fly, *Dacus vertebratus* exhibited a complete metamorphosis: egg, larva, pupa and adult in their development and also had three larval stages (Plate 4.3). Morphometric measurements of each instar of development are presented in Table 4.6. Eggs laid by female *D. vertebratus* were similarly deposited in batches of 3 to 9 eggs under the epidermis of watermelon. Eggs were white in colour measuring in average 1.02 ± 0.02 mm in length and 0.18 ± 0.00 mm in width. Egg hatched into a first instar larvae also white in colour. The morphometric measurements of the first instar was 2.74 ± 0.07 mm in length and 0.49 ± 0.04 mm in width. Second instar larvae was whitish and measured 4.58 ± 0.14 mm and 0.67 ± 0.02 mm. The third instar was yellow and jumped at mature into soil to pupate. It measured an average 7.60 ± 0.14 mm in length and 1.18 ± 0.02 mm in width. Pupae formed were slightly brown and measured 5.22 ± 0.16 mm in length and 1.97 ± 0.07 mm in width. Adult of *D. vertebratus* was brown in colour with transparent wing. The presence of an ovipositor as an extending of the abdomen of the female differentiated it from the male. Adult female of *D. vertebratus* measured in length 6.26 ± 0.27 mm and 2.08 ± 0.11 mm in width while male was bigger in size (length = 6.33 ± 0.18 mm and width = 2.16 ± 0.10 mm). The wings had distinct postero-distal corner of cell punted with an enlarged spot

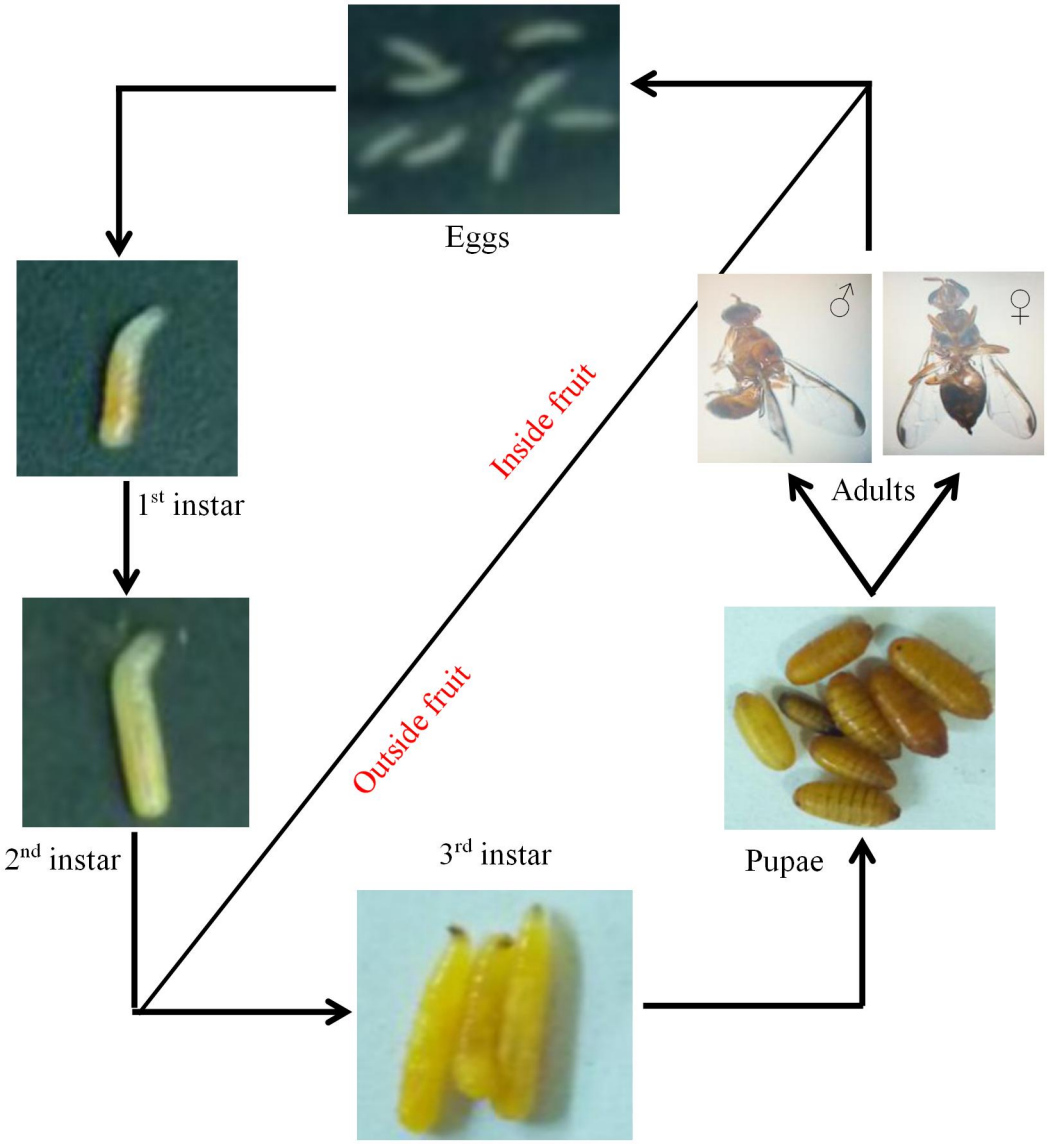


Plate 4.3. Life cycle of *Dacus vertebratus*

Magnification : x 200

at wing edge. Wings have a complete R-costal. The legs of *D. vertebratus* had bi-coloured femurs (yellowish and brown). On the thorax, the scutum was red-brown with no marking. The scutellum was yellow in colour.

4.2.3 Developmental biology of *Dacus vertebratus* and *Zeugodacus cucurbitae* on *Citrullus lanatus*

The duration of each host instar development of *D. vertebratus* and *Z. cucurbitae* is presented in Table 4.7. The eggs hatched on the 2nd and 3rd days with an average eggs incubation period 1.60 ± 0.15 days on *D. vertebratus* shorter than 1.95 ± 0.17 days on *Z. cucurbitae.* Egg hatched into the first instar and fed on the flesh of the fruit. The first instar lasted for an average time of 2.45 ± 0.11 days for *Z. cucurbitae* and 2.55 ± 0.11 days for *D. vertebratus.* The duration of the second instar development varied from of 2.60 ± 0.13 days for *Z. cucurbitae* to 2.45 ± 0.11 days for *D. vertebratus.* Third instar lasted 3.70 ± 0.15 days (*D. vertebratus*) to 4.10 ± 0.19 days (*Z. cucurbitae*). At this stage, the third instar jumped into the soil for pupation (pupal stage). It took 6.30 ± 0.23 days for the pupae to emerge into adults for *D. vertebratus* while pupal stage lasted for 7.65 ± 0.18 days for *Z. cucurbitae*. There was a significant difference in the pupal duration among fruit fly species. An average time of 16.60 ± 0.27 days was required for the female development of *D. vertebratus* which was significantly shorter than that of the female *Z. cucurbitae* (18.75 ± 0.40 days). Similarly, the duration of male of *D. vertebratus* (16.65 ± 0.31 days) is shorter than male developmental time of *Z. cucurbitae* (17.50 ± 0.34 days).

4.2.4 Survival rate of immature stages of *Dacus vertebratus* and *Zeugodacus cucurbitae*

Survival rate of each host instar development was not significantly different among fruit fly species as presented in Table 4.8. *D. vertebratus* recorded the highest first instar (72.70 ± 2.65%), second instar (92.73 ± 1.395%) and pupal survival rate (85.49 ± 2.41%) as compared to *Z. cucurbitae* (71.65 ± 2.58%), (90.64 ± 1.63) and (80.67 ± 4.02%) respectively. Conversely, percentage survival rate of eggs (79.20 ± 2.64%) and the third instar of *Z. cucurbitae* (93.06 ± 1.81%) were slightly higher than the egg survival (78.45 ± 2.45%) and third instar (92.49 ± 1.34%) of *D. vertebratus*.

Table 4.7. Duration of development of immature stages of *Zeugodacus cucurbitae* and *Dacus vertebratus*

Fruit fly species	Eggs incubation	First instar	Second instar	Third instar	Pupal period	Duration (female)	Duration (male)
Zeugodacus cucurbitae	1.95 ± 0.17a	2.45 ± 0.11a	2.60 ± 0.13a	4.10 ± 0.19a	7.65± 0.18b	18.75 ± 0.40b	17.50 ± 0.34a
Dacus vertebratus	1.60 ± 0.15a	2.55 ± 0.11a	2.45 ± 0.11a	3.70 ± 0.15a	6.30 ± 0.23a	16.60 ± 0.27a	16.65 ± 0.31a

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.8. Survival rate of immature stages of *Zeugodacus cucurbitae* and *Dacus vertebratus*

Fruit fly species	Eggs hatch	First instar	Second instar	Third instar	Pupal survival
Zeugodacus cucurbitae	79.20±2.64a	71.65±2.58a	90.64±1.63a	93.06±1.81a	80.67±4.02a
Dacus vertebratus	78.45±2.45a	72.70±2.65a	92.73±1.39a	92.49±1.34a	85.49±2.41a

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

4.2.5 Emergence rate and sex-ratio of *Dacus vertebratus* and *Zeugodacus cucurbitae*

Emergence rate and sex-ratio of *D. vertebratus* and *Z. cucurbitae* are presented in Table 4.9. *Zeugodacus cucurbitae* recorded the highest emergence rate (63.50 ± 3.10%) which was significantly different from the mean percentage of adult of *D. vertebratus* (52.00 ± 4.14%). Also *Z. cucurbitae* showed high number of emerged male from pupae as compared to number of female (1.60 ± 0.33). Sex-ratio of *D. verebratus* was 1.19 ± 0.27 showing that the number of emerged male was slightly higher than the number of emerged female. However, emergence rate and sex-ratio were not significantly different among fruit fly species.

4.3 Aspects of ecology of *Dacus vertebratus* and *Zeugodacus cucurbitae*

Ecological parameters such as the interaction between host range and temperature, adult diets and parental ages are presented below.

4.3.1 Susceptibility of eight vegetable host to *Dacus vertebratus*

4.3.11 Effect of vegetable fruits and temperatures on the fecundity of *Dacus vertebratus*

The fecundity of *D. vertebratus* was daily recorded and this varied among vegetable fruits and temperature as showen in Figure 4.4. In a controlled laboratory temperature of 30 ± 1℃, the highest number of eggs laid by a single female was recorded on magda-zucchini (723.00) followed by caserta-zucchini (709.60), pawpaw (693.60), fordhook-zucchini (686.20), melon (660.40), pickled-cucumber (655.80), cucumber (606.20) and watermelon (550.60). At 25 ± 1℃, watermelon ranked first with a total number of 650.40 eggs which was higher than that of pawpaw (537.60), fordhook-zucchini (537.40), cucumber (528.60), pickled-cucumber (528.00), melon (521.60), magda-zucchini (515.00) and caserta-zucchini (510.00). The highest number of eggs was recorded between day 4 and day 13. However, at both temperature, daily eggs record showed a reduction in number of eggs on all vegetable fruits as adults gets old.

Table 4.9. Emergence rate (%) and sex-ratio of *Zeugodacus cucurbitae* and *Dacus vertebratus*

Fruit fly species	Sex-ratio	Emergence rate
Zeugodacus cucurbitae	1.60 ± 0.33a	63.50 ± 3.10a
Dacus vertebratus	1.19 ± 0.27a	52.00 ± 4.14a

* Mean within columns followed by the same letters are not significantly different at α = 0.05

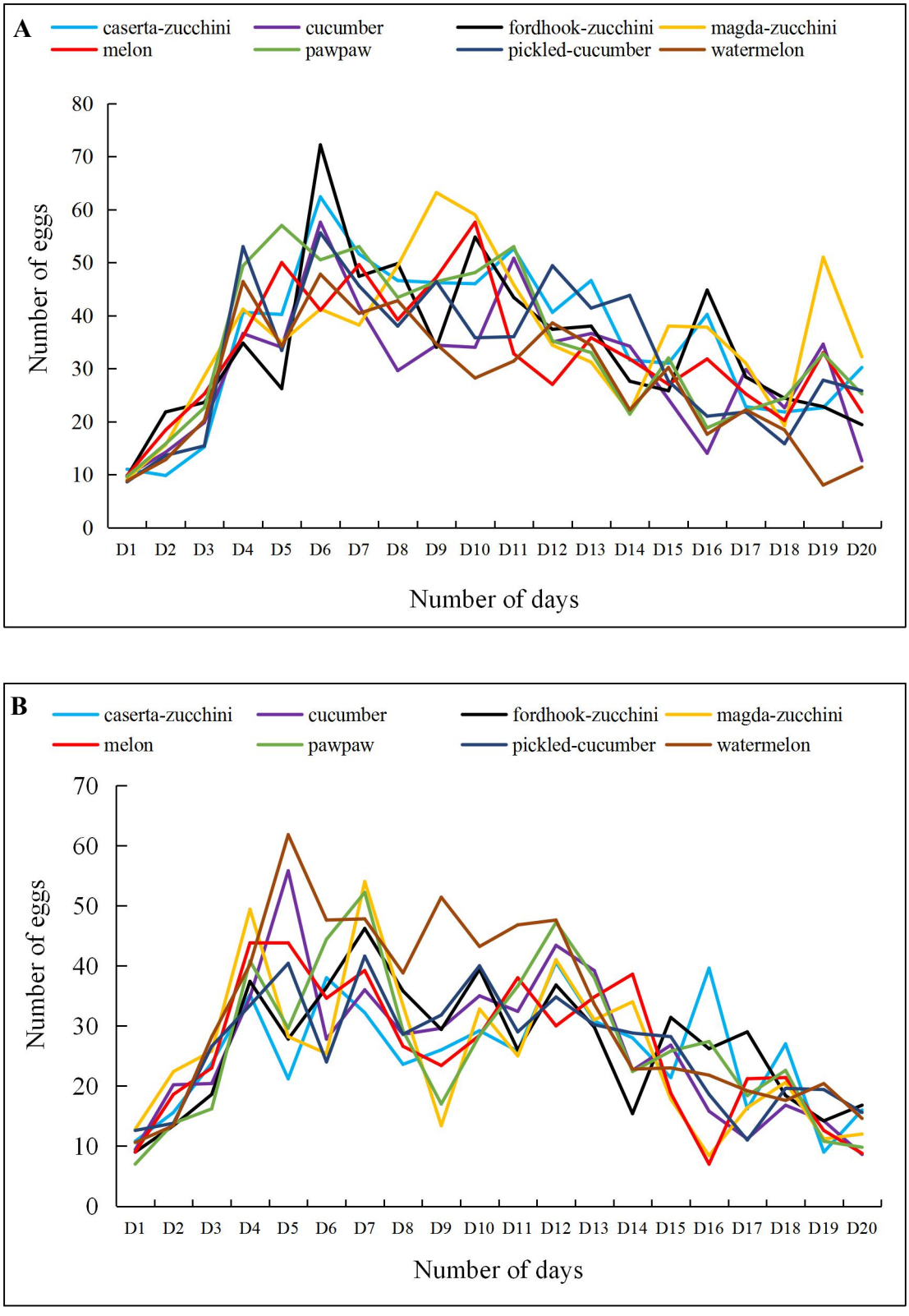


Figure 4.4. Fecundity of *Dacus vertebratus* on eight vegetable fruit at A) 30 ± 1°C and B) 25 ± 1°C

4.3.1.2 Effect of vegetable fruits and temperatures on the total development time of *Dacus vertebratus*

The development of *D. vertebratus* significantly differed from one vegetable fruit to another (Table 4.10). The shortest total development time of *D. vertebratus* was observed on magda-zucchini (15.30 ± 1.34 days) at 30 ± 1℃ and on watermelon (16.60 ± 1.26 days) at 25 ± 1℃. It took 20.00 ± 1.05 days for the fly to complete its development on melon at both temperatures. The longest development was on pawpaw (20.50 ± 1.08 days) at 30 ± 1℃. However, vegetable fruits significantly affected the total development time of *D. vertebratus* ($P < 0.05$) as opposed to temperature.

4.3.1.3 Temperature and vegetables host fruits effect on the longevity of *Dacus vertebratus* during eggs laying period

Longevity of adult male and female of *D. vertebratus* during eggs laying period was significantly ($P < 0.05$) affected by vegetable fruits as presented in Table 4.11. However, male and female longevity was not significantly different among temperatures. During egg laying period, the average duration of longevity of an adult female of *D. vertebratus* ranged from 18.07 ± 4.45 days on fordhook-zucchini to 21.27 ± 4.56 days on magda-zucchini at 25 ± 1℃. Adult female longevity lasted for 21.70 ± 3.83 days on pickled-cucumber with the shortest longevity recorded on watermelon 17.33 ± 4.22 days at 30 ± 1℃. Male of *D. vertebratus* lived longer on magda-zucchini (20.33 ± 4.24 days) and the shortest time was recorded on watermelon at both temperatures.

4.3.1.4 Temperature and vegetables host fruits effect on number of pupae of *Dacus vertebratus* and adult emergence

Susceptibility of eight vegetables to *D. vertebratus* was assessed to observe if they can support the development of its life stages. Temperature and vegetable host fruits significantly affected the number of pupae recovered ($P < 0.05$). More pupae were produced at 30 ± 1℃ regardless the vegetable fruit used as compared to 25 ± 1℃. The number of pupae are shown in Figure 4.5a and 4.5b. At 25 ± 1℃, the number of pupae collected were 3.53 ± 1.80 (caserta-zucchini), 1.28 ± 0.07 (magda-zucchini), 0.94 ± 0.56 (fordhook-zucchini), 0.30 ± 0.13 (cucumber), 0.25 ± 0.12 (picked-cucumber), 0.06 ± 0.00 (melon) and 0.03 ± 0.00 (pawpaw). The highest number of pupae was on

Table 4.10. Duration of total development time on different vegetables under two temperatures

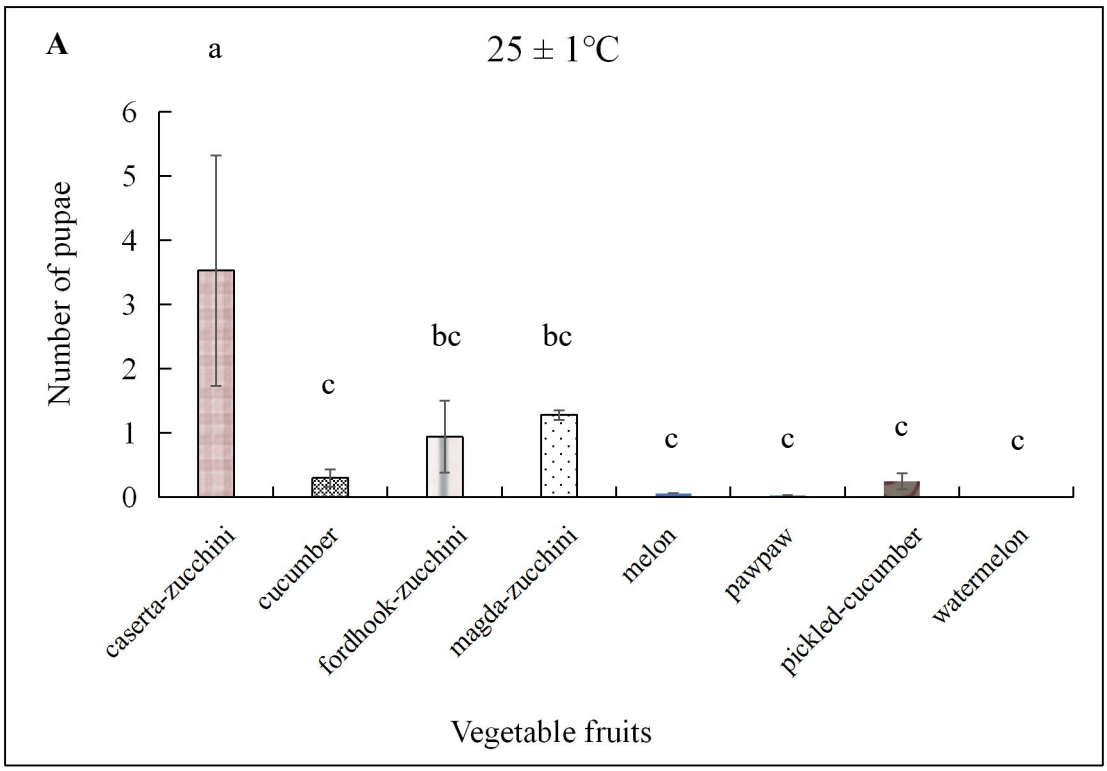
Vegetable fruits	25 ± 1°C	30 ± 1°C
Cucurbita pepo var. caserta-zucchini	18.30 ± 1.57c	18.60 ± 1.90bc
Cucumis sativa (cucumber)	19.70 ± 1.15abc	19.80 ± 1.03abc
Cucurbita pepo var. fordhook-zucchini	19.11 ± 1.36abc	19.55 ± 1.69abc
Cucurbita pepo var. magda-zucchini	16.70 ± 1.64d	15.30 ± 1.34d
Cucumis melo (melon)	20.00 ± 1.05ab	20.00 ± 3.92ab
Carica papaya (pawpaw)	19.60 ± 1.90a	20.50 ± 1.08abc
Cucumis sativa (pickled-cucumber)	20.00 ± 1.49ab	19.60 ± 2.17ab
Citrullus lanatus (watermelon)	16.60 ± 1.26d	16.40 ± 1.17d

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.11. Longevity of *Dacus vertebratus* on different vegetables under two temperatures

Vegetable fruits	Female		Male	
	25 ± 1°C	30 ± 1°C	25 ± 1°C	30 ± 1°C
Cucurbita pepo var. caserta-zucchini	20.10 ± 3.95abc	20.10 ± 3.96abc	20.27±3.11ab	20.26±3.11ab
Cucumis sativa (cucumber)	20.57 ± 3.76ab	19.70 ± 3.96abc	18.67±4.53abc	20.63±4.30a
Cucurbita pepo var. fordhook-zucchini	18.07 ± 4.45cd	18.17 ± 4.09cd	20.20±4.06ab	18.63±3.96bc
Cucurbita pepo var. magda-zucchini	21.27 ± 4.56ab	21.27 ± 4.56ab	20.33±4.24ab	20.33±4.24ab
Cucumis melo (melon)	19.37 ± 5.12	19.40 ± 5.13	19.13±3.67ab	19.17±3.70ab
Carica papaya (pawpaw)	20.13 ± 4.34abc	20.13 ± 4.35abc	19.07±3.64ab	19.07±3.64ab
Cucumis sativa (pickled-cucumber)	20.00 ± 3.61abc	21.70 ± 3.83a	20.20±3.80ab	19.70±3.31ab
Citrullus lanatus (watermelon)	18.20 ± 4.50cd	17.33 ± 4.22d	16.80±4.32cd	16.60±4.59d

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$



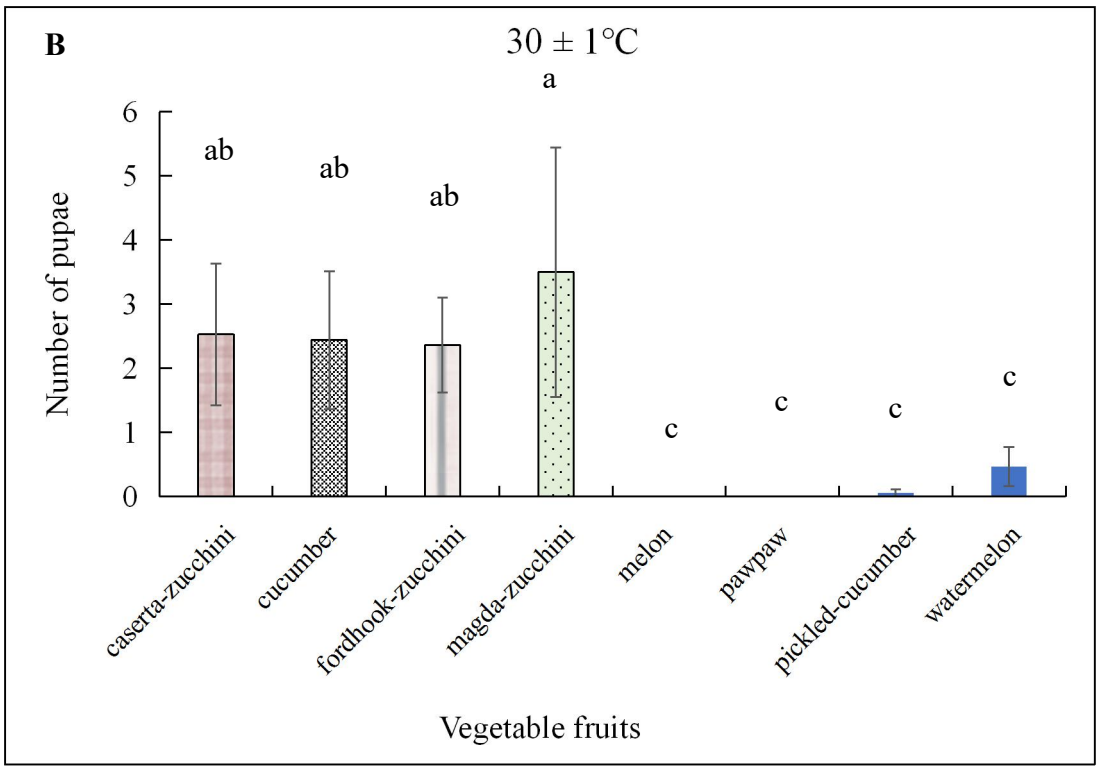


Figure 4.5. Number of pupae recovered from vegetable fruit at A) 25 ± 1°C and B) 30 ± 1°C

magda-zucchini (3.50 ± 1.94) followed by caserta-zucchini (2.53 ± 1.11), cucumber (2.44 ± 1.07), fordhook-zucchini (2.36 ± 0.74), watermelon (0.47 ± 0.31) and picked-cucumber (0.06 ± 0.05). The three varieties of zucchini were more susceptible to *D. vertebratus*. Percent emergence rate was highest on pupae collected from cucumber (23.11 ± 7.77%) at 30 ± 1℃ while it was 21.03 ± 7.69% on magda-zucchini at 30 ± 1℃ (Table 4.12).

4.3.1.5 Temperature and vegetables host fruits effect on number of male and female emerged from pupae collected from different vegetable hosts

The numbers of male and female recorded from emerged adults are presented in Table 4.13. Vegetables fruits significantly affected the numbers of female and males emerged from pupae ($P < 0.05$). At 25 ± 1℃, male recorded the highest number of 1.08 ± 0.81 while for female it was 0.75 ± 0.58 from caserta-zucchini. The lowest number of male was on pawpaw (0.03 ± 0.05) and on pickled-zucchini (0.03 ± 0.05) for female. However, at 30 ± 1℃ the number of male was the highest on caserta-zucchini (1.16 ± 0.74) followed by magda-zucchini (0.94 ± 0.81), cucumber (0.81 ± 0.55) and fordhook-zucchini (0.75 ± 0.40). As for the female, magda-zucchini recorded the highest number (0.89 ± 0.73) and the lowest was on pickled-zucchini (0.03 ± 0.05).

4.3.2 Suitability of two protein-based diets as adult fly foods for mass rear *Dacus vertebratus*

The effect of two protein-based diets made up a mixture of mixture soybean + white sugar and mixture of yeast + brown sugar was assessed in the laboratory. The results are presented as follows:

4.3.2.1 Effect of adult diet on the fecundity of *Dacus vertebratus*

Adult's food made up two protein-based diets had no significant effect on the fecundity of *D. vertebratus* as shown in Figure 4.6. The highest number of eggs were recorded from adults fed with the mixture of yeast and brown sugar (568.00) as compared to the number of eggs laid by flies fed with mixture of soya-bean and white sugar (533.00). Daily eggs collection registered the highest number of eggs the second day with both diet. Daily number of eggs varied from 20.00 ± 1.95 to 58.20 ± 3.43 when flies were fed with mixture of yeast and brown sugar. When flies fed on the mixture of soya-bean and white sugar, it ranged from 14.80 ± 1.02 to 51.60 ± 2.29.

Table 4.12. Emergence rate (%) of *Dacus vertebratus* on different vegetables under two temperatures

Vegetable fruits	25 ± 1°C	30 ± 1°C
Cucurbita pepo var. caserta-zucchini	19.95 ± 7.60 abc	21.30 ± 7.60 a
Cucumis sativa (cucumber)	16.67 ± 8.45 abcd	23.11 ± 7.77 a
Cucurbita pepo var. fordhook-zucchini	8.65 ± 4.50 cdef	21.71 ± 6.92 a
Cucurbita pepo var. magda-zucchini	21.03 ± 7.69 ab	12.37 ± 5.51 abcde
Cucumis melo (melon)	0.00 ± 0.00 f	0.00 ± 0.00 f
Carica papaya (pawpaw)	2.78 ± 3.73 ef	0.00 ± 0.00 f
Cucumis sativa (pickled-cucumber)	9.72 ± 6.45 bcdef	2.78 ± 3.73 ef
Citrullus lanatus (watermelon)	0.00 ± 0.00 f	7.22 ± 4.97 def

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.13. Number of male and female of *Dacus vertebratus* emerged from different vegetables under two temperatures

Vegetable fruits	Male		Female	
	25 ± 1℃	30 ± 1℃	25 ± 1℃	30 ± 1℃
Cucurbita pepo var. caserta-zucchini	1.08 ± 0.81a	1.16 ± 0.74	0.75 ± 0.58a	0.61 ± 0.42a
Cucumis sativa (cucumber)	0.11 ± 0.12c	0.81 ± 0.55ab	0.14 ± 0.13a	0.81 ± 0.52a
Cucurbita pepo var. fordhook-zucchini	0.33 ± 0.29bc	0.75 ± 0.40b	0.25 ± 0.21a	0.53 ± 0.27a
Cucurbita pepo var. magda-zucchini	0.31 ± 0.18bc	0.94 ± 0.81a	0.28 ± 0.21a	0.89 ± 0.73a
Cucumis melo (melon)	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
Carica papaya (pawpaw)	0.03 ± 0.05c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
Cucumis sativa (pickled-cucumber)	0.11 ± 0.10c	0.00 ± 0.00c	0.03 ± 0.05b	0.03 ± 0.05b
Citrullus lanatus (watermelon)	0.00 ± 0.00c	0.17 ± 0.16c	0.00 ± 0.00b	0.11 ± 0.13b

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

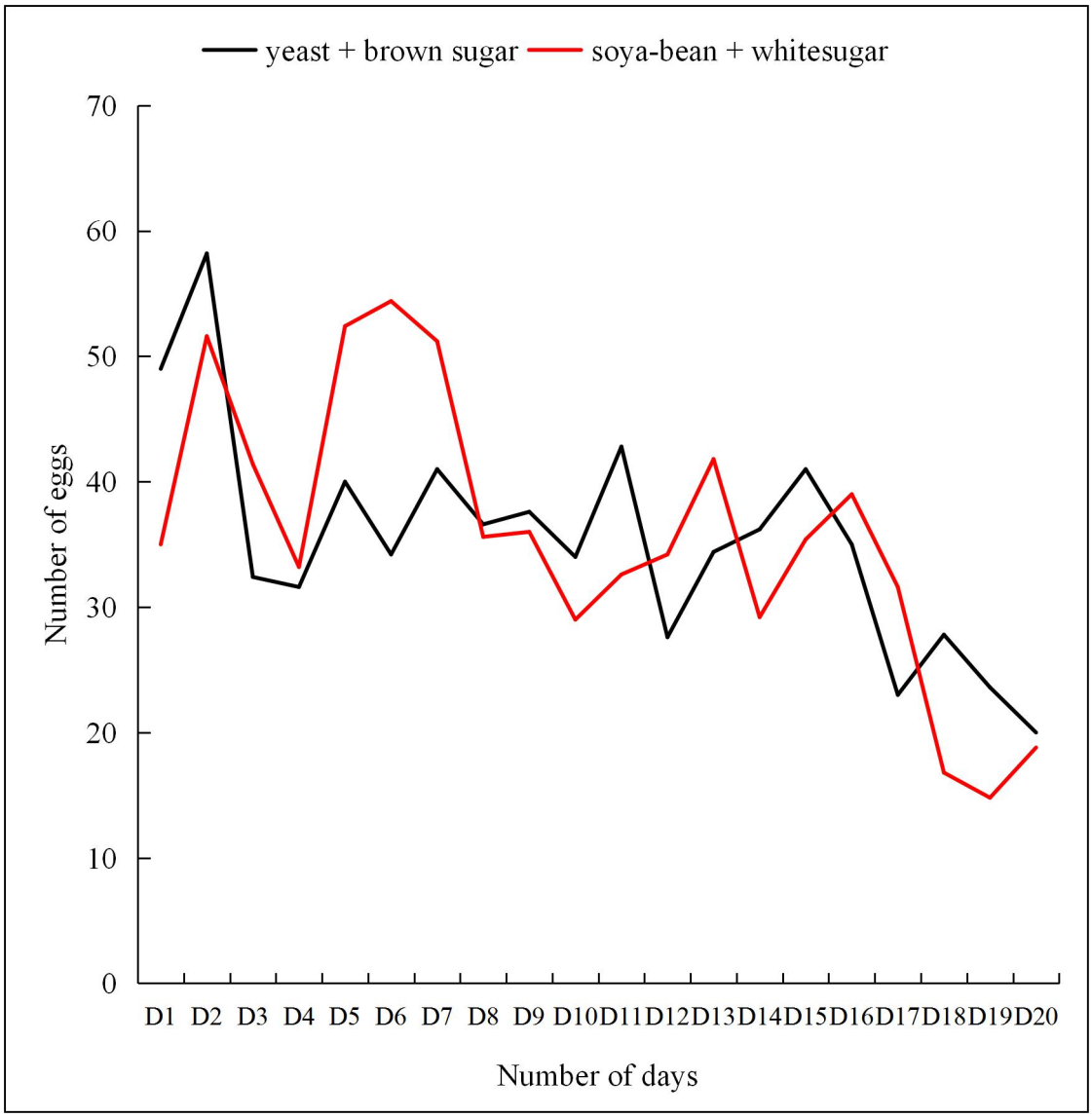


Figure 4.6.Fecundity of *Dacus vertebratus* from adults fed with two protein-based diets

4.3.2.2 Effect of adult diet on the total developmental time of *Dacus vertebratus*

The development time of *D. vertebratus* emerged after feeding of parents on two different diets is shown in Figure 4.7. The shortest development time (18.20 ± 0.39 days) was recorded from adults fed with the mixture of yeast and brown sugar. Development time was longer on offspring produced by adult flies fed with the mixture soybean + white sugar (18.40 ± 0.47 days). However, *D. vertebratus* developed faster when parents were young (12 to 16 days old). However, no significant difference was observed in the total development time of *D. vertebratus* when fed with different adults diets.

4.3.2.3 Effect of feeding of adult on number of pupae and adult emergence of *Dacus vertebratus*

The highest number of pupae was collected from fruits infested by adults fed with the mixture of yeast and brown sugar (106.83) and the lowest with mixture of soya-bean + white sugar (88.50). The number of pupae decreased as adults fed with soya-bean + white sugar gets mature as shown in Figure 4.8. However, the number of collected pupae produced by adults fed with both diets was not significantly affected by both adult diets and parental age. The highest number of pupae (25.83 ± 5.97) was collected when adults fed with yeast and brown sugar had 12 days old while adults fed with soya-bean + white sugar gave the highest pupae number (20.17 ± 4.17) at 16 days old.

4.3.2.4 Effect of adult diet and parental age on percent emergence rate and sex-ratio of *Dacus vertebratus*

Emergence of adults is presented in Table 4.14. Adult diets and parental age had no significant effect on the emergence rate of *D. vertebratus* ($P > 0.05$). Adult fed with the mixture of yeast and brown sugar produced pupae with highest emergence rate at parental age of 16 days (60.31 ± 7.01%) to 66.47% ± 0.02) followed by 20 days (57.67 ± 8.42%), 12 days (57.50 ± 6.57%), 14 days (54.96 ± 10.75%) and 22 days (41.54 ± 9.45%). When fed with soya-bean + white sugar, emergence rate was high at parental age of 18 days (60.83 ± 8.33%) with the lowest recorded at 22 days (40.64 ± 11.73%).

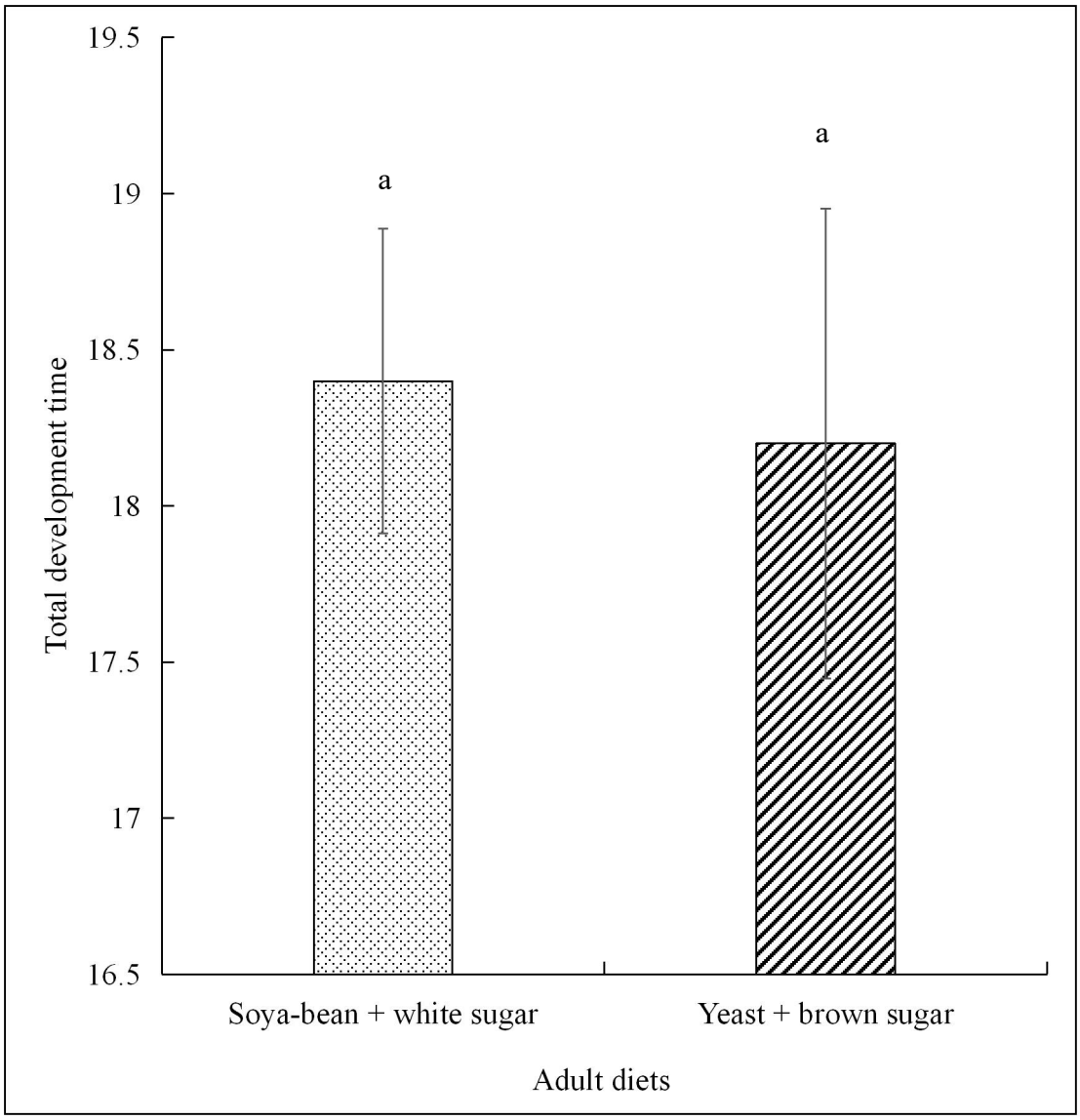


Figure 4.7. Duration of total development of *Dacus vertebratus* from adults fed with two protein-based diets

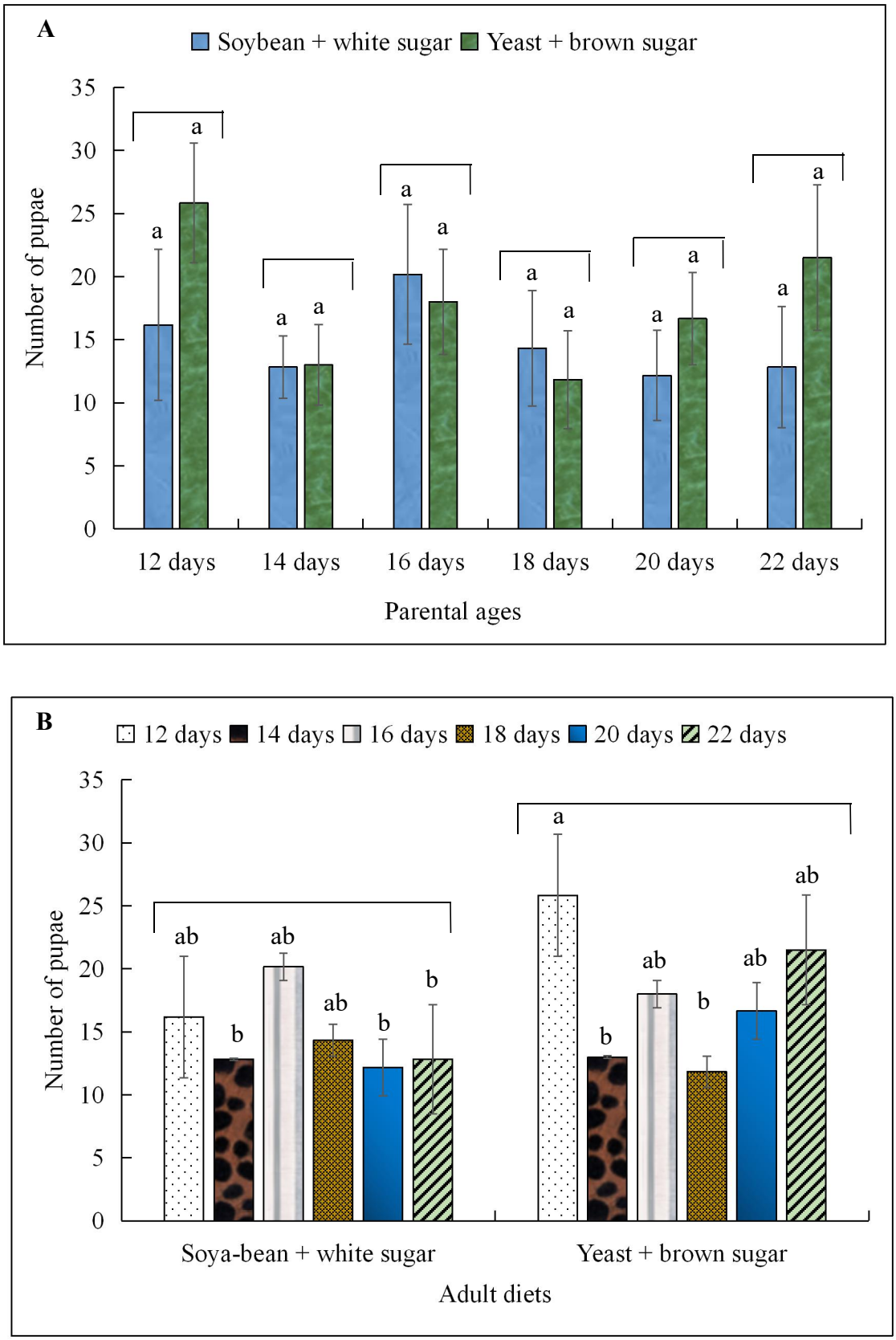


Figure 4.8. Effect of A) parental age and B) different diets fed by adult flies on number of pupae

Table 4.14. Emergence (%) of adults of *Dacus vertebratus* from pupae produced by adults fed with different diets

Parental ages	Mixture of soya-bean + white sugar	Mixture of yeast + brown sugar
12 days	55.23 ± 7.96a	57.50 ± 6.57a
14 days	54.16 ± 8.97a	54.96 ± 10.75a
16 days	48.11 ± 9.43a	60.31 ± 7.01a
18 days	60.83 ± 8.33a	48.46 ± 10.46a
20 days	49.80 ± 10.31a	57.67 ± 8.42a
22 days	40.64 ± 11.73a	41.54 ± 9.45a

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

The ratio of male and female that emerged from pupae produced by adults fed with different diets is presented in Table 4.15. Sex-ratio was significantly higher than one at parental age of 14 days (1.25 ± 0.27), 16 days (1.05 ± 0.21), 18 days (1.79 ± 0.42) and 20 days (1.18 ± 0.38) showing that more males emerged from pupae produced by adults fed with soya-bean and white sugar. From pupae produced by adults fed with yeast and brown sugar, the number of emerged females were higher than the number of males at parental age of 14 days (0.87 ± 0.25), 18 days (0.98 ± 0.22) and 22 days (0.89 ± 0.29). However, parental age significantly affected the sex-ratio of *D. vertebratus* ($P > 0.05$).

4.3.3 Development of *Zeugodacus cucurbitae* on three vegetables host fruits in choice and no choice conditions

The effect of three vegetable fruits *Cucurbita pepo*, *Citrullus lanatus* and *Carica papaya* on the development of *Z. cucurbitae* in terms of immature instar development, pupal production, weight and morphometric are as follows :

4.3.3.1 Effect of vegetables host fruits on larval, pupal development time of *Zeugodacus cucurbitae*

The duration of each developmental stage of *Z. cucurbitae* is presented in Table 4.16. The longest larval development time was on *Cucurbita pepo* in both no-choice and choice tests (6.13 ± 0.13 days and 6.09 ± 0.09 days respectively). The shortest duration was recorded on *Citrullus lanatus* (5.91 ± 0.09 days). However, larval development time was not significantly different among vegetable host fruits and the tests in which parents were found.

In choice condition, pupal development times (pupae to adult emergence) were shortest on *Cucurbita pepo* (6.97 ± 0.12 days) while in no-choice tests the longest development time was 9.15 ± 0.27 days on the same vegetable host fruit. Both vegetable host fruits ($P < 0.05$) and the conditions ($P < 0.05$) significantly affected pupal development time. There was also an interaction between the conditions and vegetable host fruits ($P < 0.05$).

Total development time of *Z. cucurbitae* significantly varied among vegetable host fruits ($P < 0.05$). The fly developed faster on *Citrullus lanatus* (12.83 ± 0.13 days) in choice tests, with the longest development time on *Carica papaya* (13.83 ± 0.14 days).

Table 4.15. Sex-ratio of emerged adults of *Dacus vertebratus* from pupae produced by adults fed with different diets

Parental ages	Mixture of soya-bean + white sugar	Mixture of yeast + brown sugar
12 days	0.78 ± 0.19c	1.61 ± 0.26ab
14 days	1.25 ± 0.27abc	0.87 ± 0.25bc
16 days	1.05 ± 0.21abc	1.15 ± 0.18abc
18 days	1.79 ± 0.42a	0.98 ± 0.22bc
20 days	1.18 ± 0.38abc	1.01 ± 0.16bc
22 days	0.92 ± 1.29bc	0.89 ± 0.29bc

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.16. Duration of immature stages of *Zeugodacus cucurbitae* as influenced by three vegetables fruits

Vegetable fruits	Choice test			No choice test		
	Larval duration	Pupal duration	Total development	Larval duration	Pupal duration	Total development
Carica papaya (pawpaw)	5.93 ± 0.096a	7.4 ± 0.13a	13.83 ± 0.14a	6.01 ± 0.13a	7.95 ± 0.23b	13.90 ± 0.25a
Citrullus lanatus (watermelon)	5.91 ± 0.095a	7.12 ± 0.12a	12.83 ± 0.13a	5.96 ± 0.18a	7.45 ± 0.22b	14.00 ± 0.25a
Cucurbita pepo (magda-zucchini)	6.09 ± 0.098a	6.97 ± 0.12a	13.23 ± 0.14a	6.13 ± 0.13a	9.15 ± 0.27a	14.75 ± 0.27a

* Mean within columns followed by the same letters are not significantly different at $\alpha = 0.05$

In no-choice test, the total development time was longer when reared on all vegetable host fruits (13.90 ± 0.25 days on *Carica papaya* and 14.75 ± 0.27 days on *Cucurbita pepo*. Total development time (egg to adults) was however affected by both vegetable host fruits (P<0.05) and the conditions (choice or no choice) (P < 0.05).

4.3.3.2 Effect of vegetable host fruits on pupal production, weight, width and length of pupae of *Zeugodacus cucurbitae*

The number of pupae produced by *Z. cucurbitae* significantly (P < 0.05) varied among vegetable host fruits as shown in Figure 4.9. The highest number of pupae collected from *Cucurbita pepo* in choice and no-choice tests was 63.06 ± 11.38 and 115.79 ± 27.99 pupae respectively. However, the lowest number of pupae was recorded on *C. papaya* in choice test (25.61 ± 4.70) and in no-choice test (47.52 ± 17.36). Pupal production was significantly different among vegetable host fruits (P < 0.05) and the conditons (choice and no-choice) (P < 0.05).

The weight of 10 pupae collected from different vegetable host fruits significantly varied among hosts as shown in Figure 4.10. In choice tests, the weight of pupae collected from *Citrullus lanatus* (0.14 ± 0.00g) was higher than the weight of pupae collected from the *Cucurbita pepo* (0.09 ± 0.00g) and *Carica papaya* (0.05 ± 0.00g). Similarly, in no-choice test, the highest pupae weight was recorded on *C. lanatus* (0.15 ± 0.01g) followed by *C. papaya* (0.11 ± 0.01g) and *C. pepo* (0.05 ± 0.00g). The weight was significantly different among vegetable host fruits (P < 0.05) and depended on the condition (P < 0.05).

The morphometric measurements of pupae collected from different vegetable fruits are presented in Table 4.17. In terms of pupal length and width, pupae collected from *Citrullus lanatus* ranked first in terms of length (0.68 ± 0.03 cm and 0.66 ± 0.02 cm) and width (0.24 ± 0.01 cm and 0.27 ± 0.02 cm) respectively in choice and no choice tests. Both pupae width and length were significantly affected by the host fruits (P < 0.05).

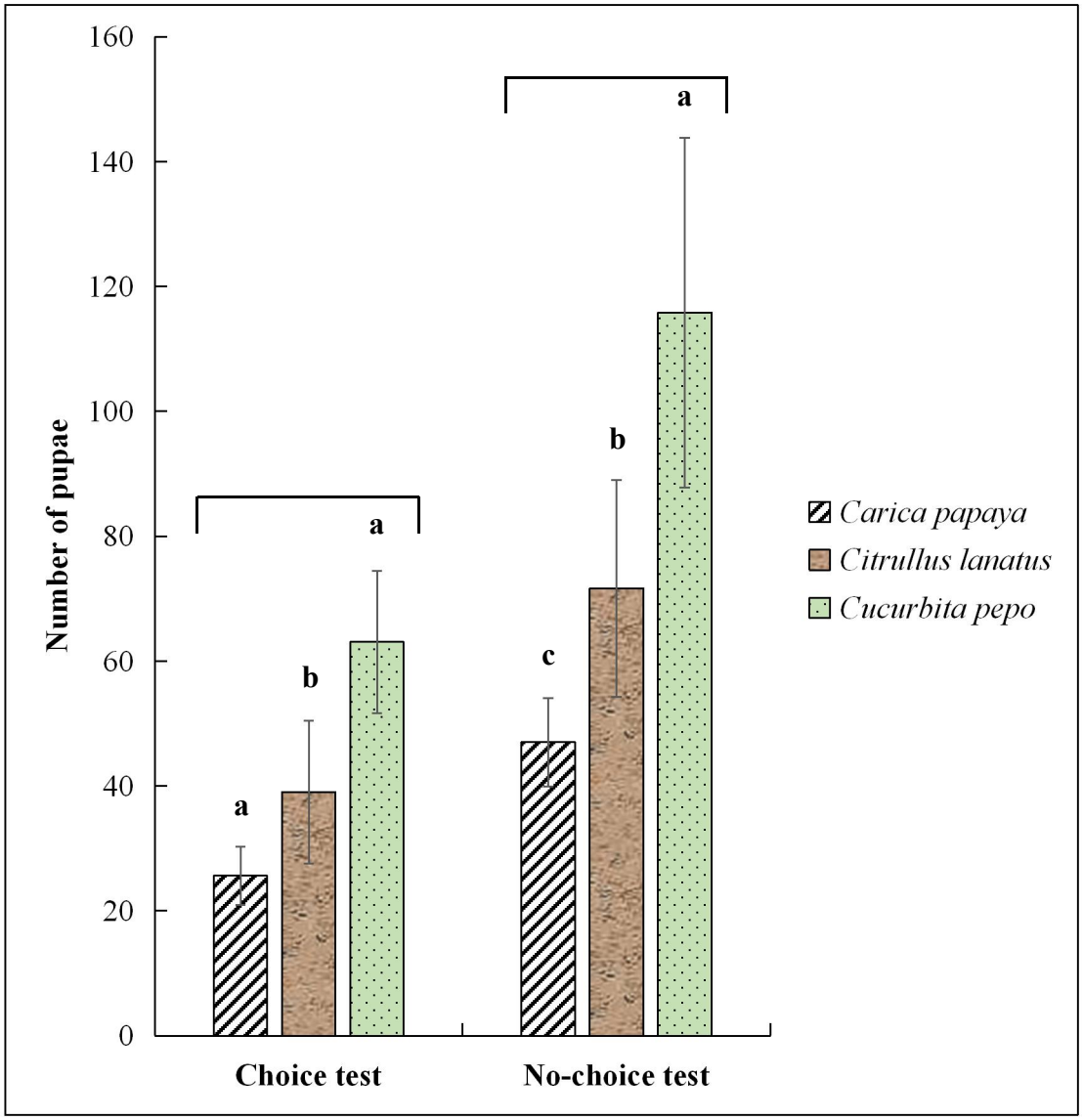


Figure 4.9. Effect of vegetable host fruits on pupae production of *Zeugodacus cucurbitae*

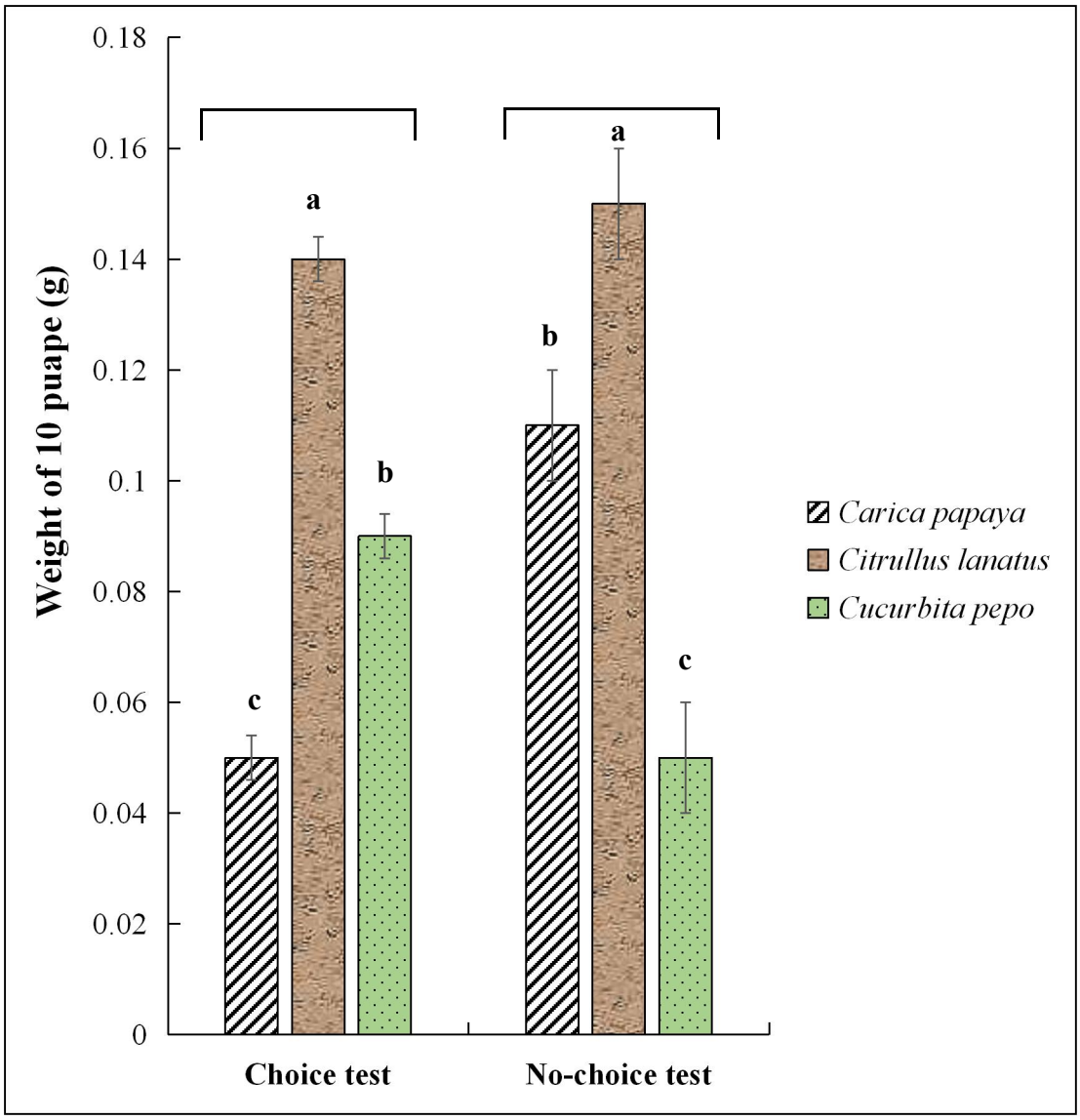


Figure 4.10. Effect of vegetable host fruits on the weight of *Zeugodacus cucurbitae*

Table 4.17. Length and width of pupae of *Zeugodacus cucurbitae* collected from three vegetable fruits

Vegetable fruits	No choice condition		Choice condition	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)
Carica papaya (pawpaw)	0.45 ± 0.03a	0.20 ± 0.02a	0.26 ± 0.02a	0.11 ± 0.01a
Citrullus lanatus (watermelon)	0.68 ± 0.03b	0.24 ± 0.01a	0.66 ± 0.02b	0.27 ± 0.02b
Cucurbita pepo (magda-zucchini)	0.37 ± 0.03a	0.20 ± 0.02a	0.44 ± 0.02a	0.21 ± 0.01b

* Mean within columns followed by the same letters are not significantly different at α = 0.05

4.3.3.3 Effect of vegetable host fruits on emergence of adults and sex-ratio of *Zeugodacus cucurbitae*

Percent adult emerged from different vegetable fruits as well as the sex-ratio are presented in Table 4.18. In both choice and no-choice tests, *Citrullus lanatus* favoured the highest adult emergence rate with an average of 58.17 ± 2.01% and 69.00 ± 3.27% respectively. The lowest emergence rate was observed on *Cucurbita pepo* (14.00 ± 2.45%) in no-choice test while it was recorded on *Citrullus lanatus* (21.50 ± 1.67%) in choice tests. There was a significant difference for emergence of adults rate between vegetable host fruits ($P < 0.05$).

Regarding the sex-ratio, more male adults emerged from *Citrullus lanatus* than females in both choice and no-choice tests (0.65 ± 0.02 and 0.68 ± 0.03 respectively). *Carica papaya* favoured more female emergence (0.26 ± 0.02) in choice test while the number of female was higher on *Cucurbita pepo* (0.37 ± 0.03) in no-choice test. However, sex-ratio showed no significant differences among vegetable host fruits and was significantly affected by the tests ($P < 0.05$).

4.3.3.4 Effect of vegetable host fruits on the flying ability of adult of *Zeugodacus cucurbitae*

Percentage of adults capable of flying is presented in Table 4.19. Flies emerged from pupae collected in no-choice test showed the highest flying ability on *Cucurbita pepo* (99.01 ± 0.01%) followed by *Carica papaya* (98.60 ± 0.01%) and *Citrullus lanatus* (98.39 ± 0.01%). In choice test, percent flying ability was not significantly lower than in no-choice test for *C. lanatus* (95.20 ± 0.02%), *C. papaya* (95.80 ± 0.02%) and *C. pepo* (96.99 ± 0.01%). Flying ability of *Z. cucurbitae* was not significantly affected by vegetable host fruits and the condition.

4.4 Efficacy of fungi with entomopathogenic potential for control of fruit flies on *Citrullus lanatus*

4.4.1 Viability of isolates of *Beauveria bassiana* and *Metarhizium anisopliae*

Out of 45 isolates screened and checked for viability, only six isolates of *B. bassiana* (Bb338, Bb353, Bb13; Bb337, Bb339 and Bb14) and one isolate of *M. anisopliae* (Ma31) were viable (Plate 4.4).

Table 4.18. Adult emergence rate (%) and sex-ratio of *Zeugodacus cucurbitae* on three vegetables host fruits

Vegetable fruits	Emergence rate		Sex-ratio	
	Choice	No choice	Choice	No choice
Carica papaya (pawpaw)	21.50 ± 1.67 a	40.50 ± 3.47 a	0.26 ± 0.02 a	0.45 ± 0.03 a
Citrullus lanatus (watermelon)	58.17 ± 2.01 b	69.00 ± 3.27 b	0.65 ± 0.02 a	0.68 ± 0.03 a
Cucurbita pepo (magda-zucchini)	55.50 ± 2.03 b	14.00 ± 2.45 c	0.44 ± 0.02 a	0.37 ± 0.03 a

*Means within rows followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.19. *Zeugodacus cucurbitae* with flying ability (%) from three different vegetables host fruits under choice and no choice conditions

Vegetable fruits	Flying adults (%)	
	Choice condition	No choice condition
Carica papaya (pawpaw)	95.80 ± 0.02 a	98.60 ± 0.01 a
Citrullus lanatus (watermelon)	95.20 ± 0.02 a	98.39 ± 0.01 a
Cucurbita pepo (magda-zucchini)	96.99 ± 0.01 a	99.01 ± 0.01 a

* Means within rows followed by the same letters are not significantly different at α = 0.05

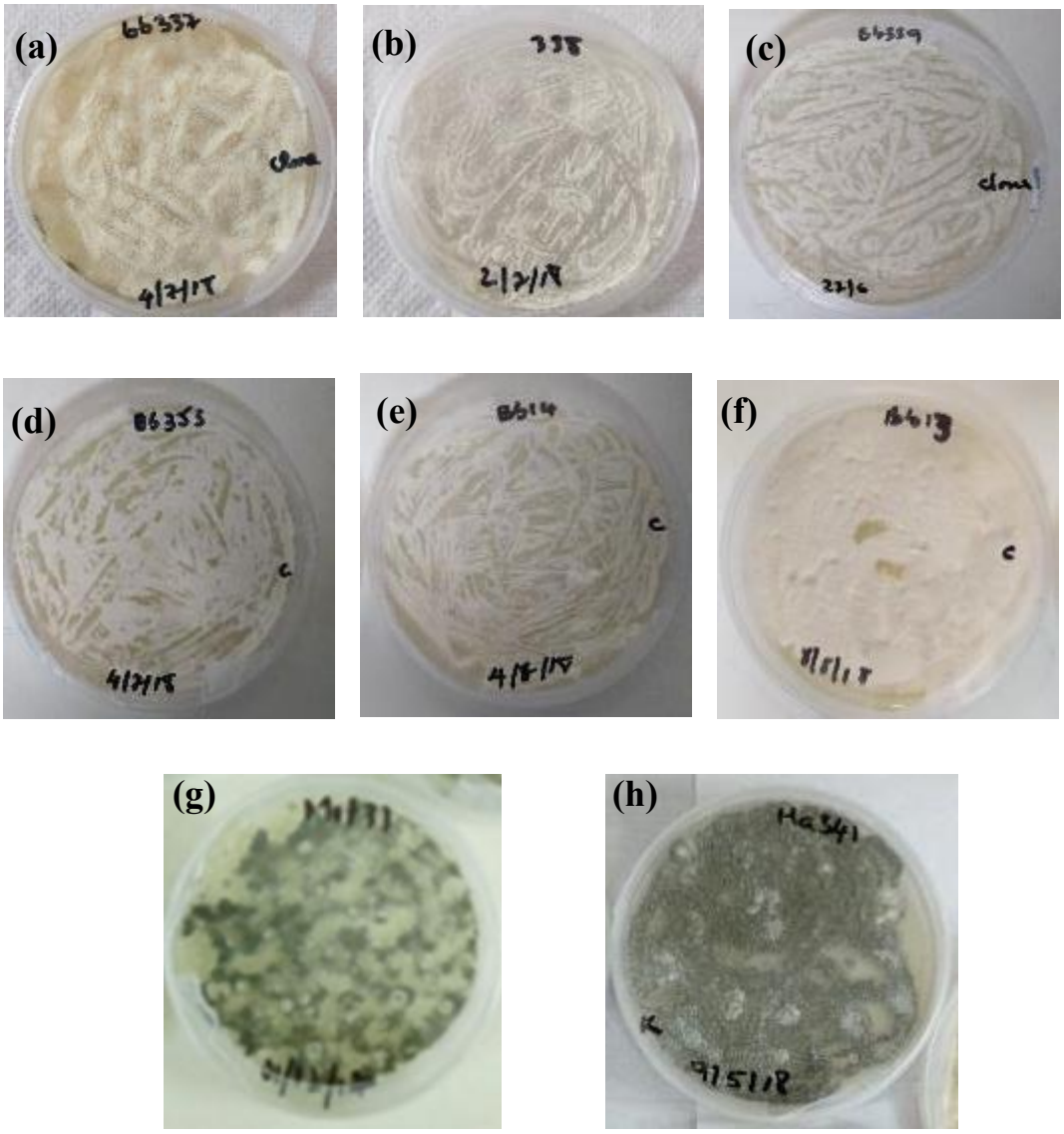


Plate 4.4. Viable isolates of *Beauveria bassiana* and *Metharhizium anisopliae* produced on artificial media (PDA)

a = Plate culture of *B. bassiana* isolate 337
b = Plate culture of *B. bassiana* isolate 338
c = Plate culture of *B. bassiana* isolate 339
d = Plate culture of *B. bassiana* isolate 353
e = Plate culture of *B. bassiana* isolate 13
f = Plate culture of *B. bassiana* isolate 14
g = Plate culture of *M. anisopliae* isolate 31
h = Plate culture of *M. anisopliae* isolate 341

The conidial germination rate for these isolate was as follow: MaD, 90.91± 2.32%; Bb14, 91.30 ± 2.94%; Ma31, 90.91 ± 2.32%; MaD, 91.30 ± 2.94%; Bb14, 92.67 ± 1.23%; Bb339, 95.54 ± 0.72%; Bb337, 95.76 ± 0.93%; Bb13, 96.73 ± 0.70%; Bb353, 97.56 ± 0.48% and Bb338, 97.76 ± 0.67%.

4.4.2 Conditioning of the entomopathogenic fungi isolates on *Zeugodacus cucurbitae*

Out of the eight isolates of *B. bassiana* and *M. anisopliae*, three isolates were recorded as the most virulent in terms of mortality and fungal growth on host instar cadavers. Percent mortality of *Z. cucurbitae* larvae and enclosed pupae is shown in Figure 4:11. It was found that percent larvae mortality caused by Bb337 (44.00±2.12%) was the highest compared to that of Bb338 (35.00±1.61%) and MaD (30.50±4.57%), with the lowest mortality percentage found in Bb339 (11.50±2.26%). However, all isolates induced high pupae mortality which ranged from 83.07±2.73% (Ma31) to 98.47±0.88% (Bb13). Percent larvae mortality was significantly different among isolates ($P<0.05$).

Fungal growth after inoculation with different isolates is showed in Figure 4.12. The highest percentage of larvae cadavers with spores was recorded with Bb337 (59.50±2.04%) followed by Bb338 (52.00±2.04%) and MaD (33.50±3.78%) with the least percentage in Ma31 (30.45±2.73%). Similarly, cadavers of pupae with mycosis was significantly high in Bb337 (65.09±2.03%) and Bb338 (40.05±1.99%) as compared to Ma31 (20.38±2.73%) and MaD (20.33±3.78%). Significant differences were observed among isolates in terms of percent larvae and pupae cadavers with mycosis ($P < 0.05$). The three isolates Bb337, Bb338 and MaD were used for bioassays.

4.4.3 Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on larvae and pupae mortality underlarval inoculation method

All isolates caused a significant mortality on larvae and enclosed pupae of *Z. cucurbitae* after larval treatment with different concentrations. Highest mortality rate was recorded with Bb337 (21.71 ± 3.45%) followed by Bb339 (17.14 ± 3.05%) and Bb337 (16.72 ± 3.01%) at 10^6 conidia/mL. The lowest larvae mortality rate was in *M. anisopliae* isolate MaD applied at all concentrations (Figure 4.13). Mortality recorded in larvae was significantly different among isolates and concentrations ($P < 0.05$).

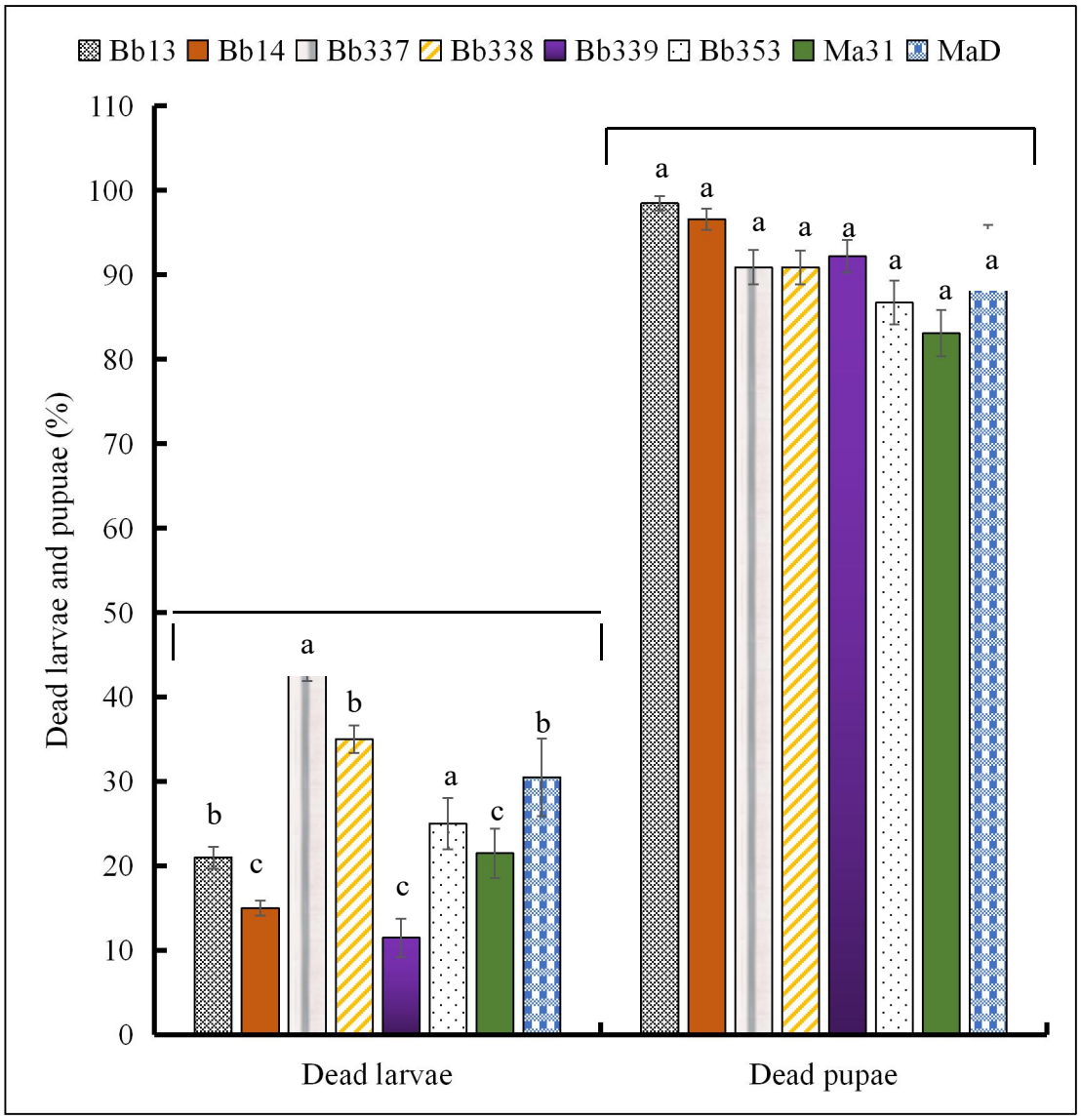


Figure 4.11. Larval and pupae of *Zeugodacus cucurbitae* mortality (%) after inoculation with isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) during the conditioning test

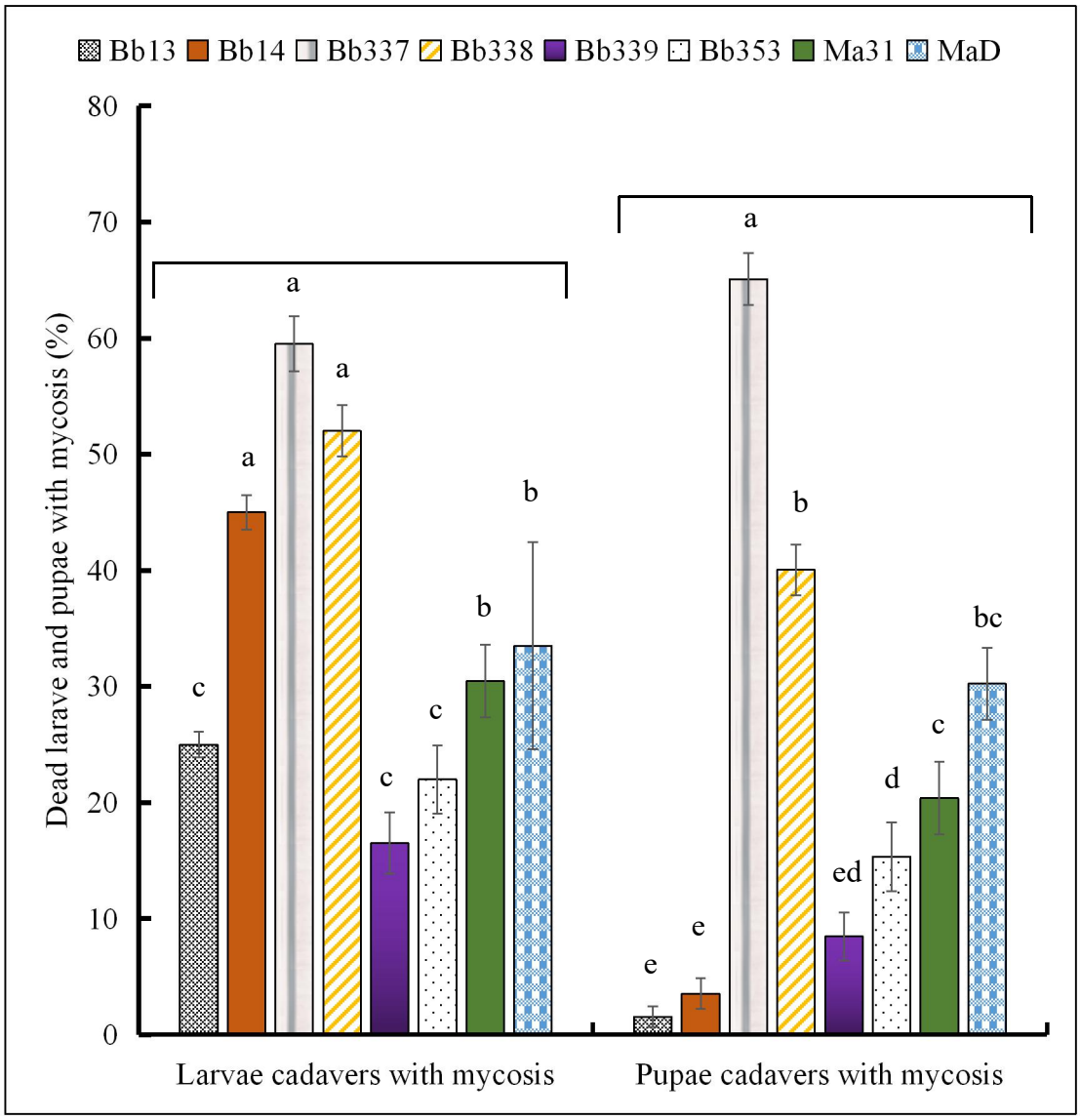


Figure 4.12. Cadavers (%) of *Zeugodacus cucurbitae* larvae and pupae after inoculation with isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) during the conditioning test

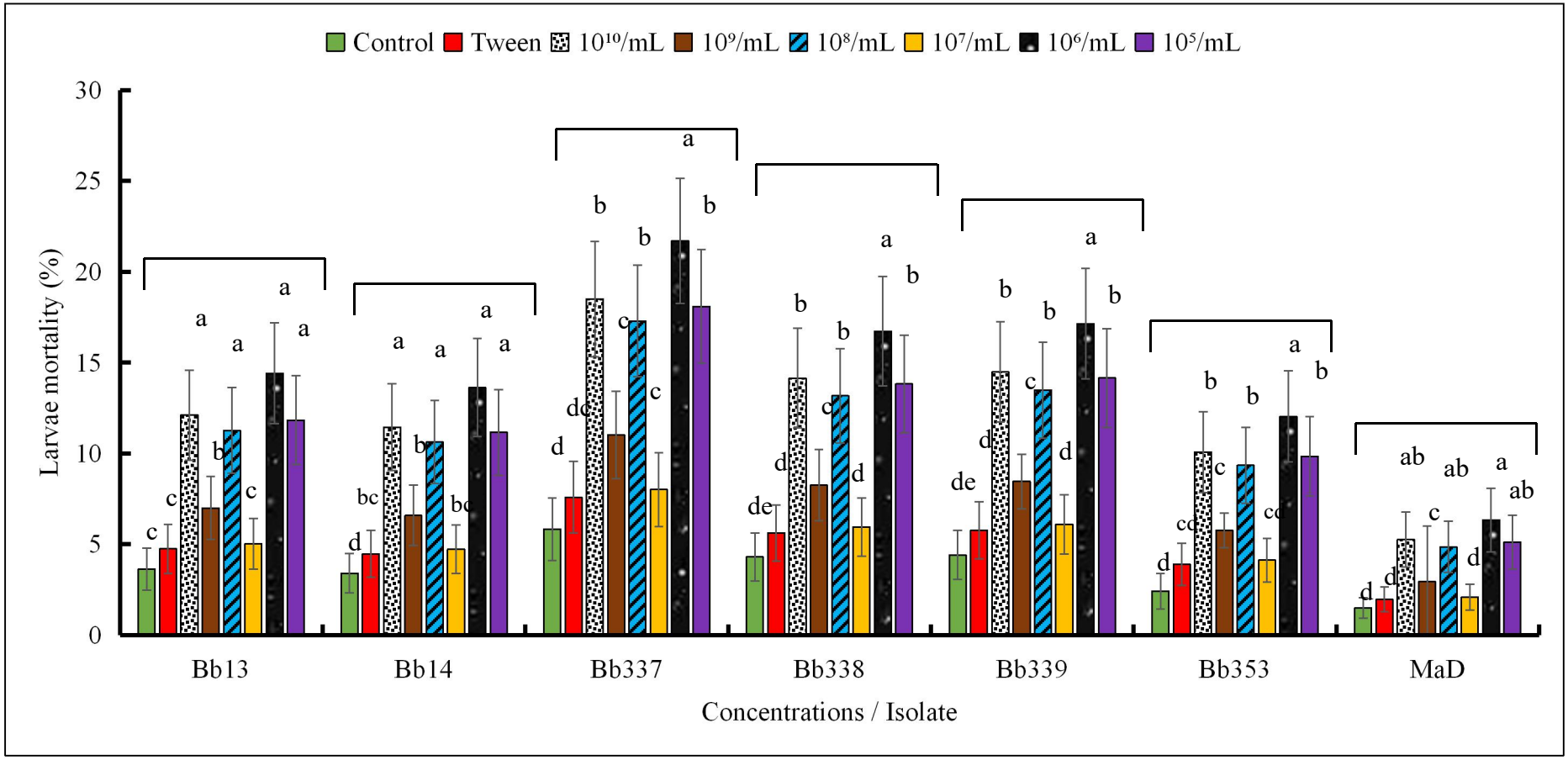


Figure 4.13. Mortality (%) of larvae of *Zeugodacus cucurbitae* treated with different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under larval inoculation method

Significant mortality was observed after 15 days of treatment in all tested concentrations. MaD was ranked first in pupae mortality rate with 63.10±3.55% when applied at 10^{10} conidia/mL followed by Bb338 (49.17 ± 3.87%) and Bb337 (47.49 ± 3.86%). Similarly, at 10^5 conidia/mL, pupae mortality rate was in order: MaD (49.29 ± 3.9%) > Bb338 (35.48 ± 3.67%) > Bb337 (33.95 ± 3.7%). The mean percent pupae mortality decreased with a decrease in conidia concentrations for all isolates (Figure 4.14). Pupae mortality was significantly different among isolates ($P < 0.05$) and concentrations ($P < 0.05$).

4.4.4 Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on adult emergence under larval inoculation method

All isolates caused a significant reduction in adult emergence (Figure 4.15) and a corresponding large mortality on pupae. Emergence rate slightly decreased with an increase in conidia concentrations of different isolates. Highest emergence rate was observed in both controls. There was a significant difference in adult emergence rate recorded among isolates ($P < 0.05$) and concentrations ($P < 0.05$).

4.4.5 Cadavers of larvae, pupae and adults with spores of *Beuaveria bassiana* and *Metarhizium anisopliae* under larval inoculation method

Cadavers of *Z. cucurbitae* with mycosis after treatment with different isolates at different concentrations is presented in Figure 4.16. Percentage cadavers with fungal growth after death is shown in Plate 4.5. Percent larvae cadavers with mycosis after infection by Bb337 and Bb338 were significantly higher than the rates recorded on others isolates. These rates were consistent and decreased only slightly with the concentrations. However, there was an increase in the percent larvae cadavers with mycosis at low concentrations (38.00 ± 0.41% and 30.80 ± 1.62%) for Bb338 when applied at 10^7 conidia/mL and 10^5 conidia/mL per insect were applied, respectively.

Similarly, a percent larvae cadavers with mycosis of 61.10 ± 2.54% was recorded for Bb337 at very low concentration 10^5 conidia/mL per insect. Percent larvae cadavers with mycosis was significantly different among concentrations ($P < 0.05$) but not among isolates.The effect of isolates on enclosed pupae (formed after larvae infection) showed high percent pupae cadavers with mycosis for all isolates when applied at 10^{10} conidia/mL per insect.

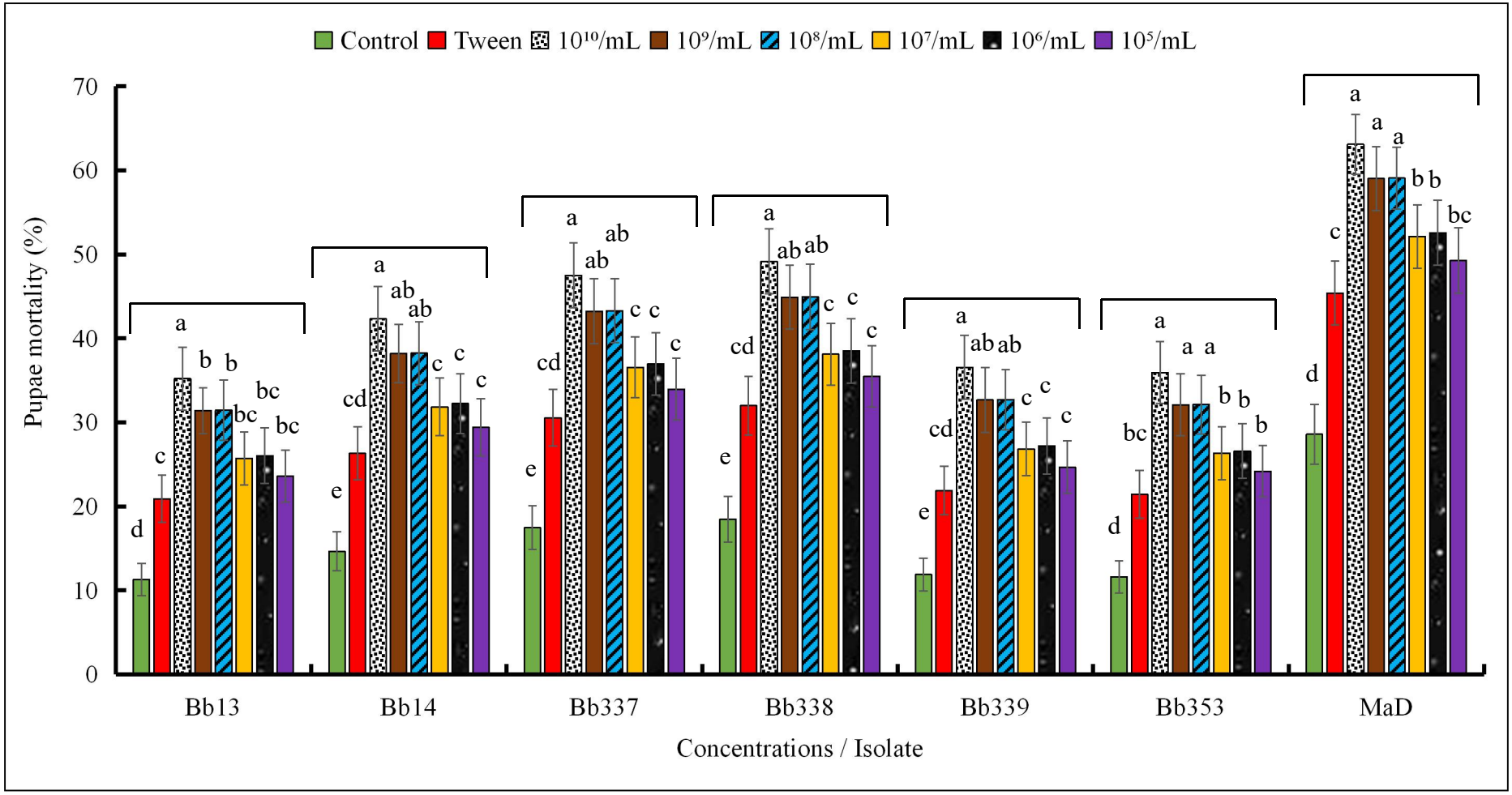


Figure 4.14. Mortality of pupae (%) of *Zeugodacus cucurbitae* treated with different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under larval inoculation method

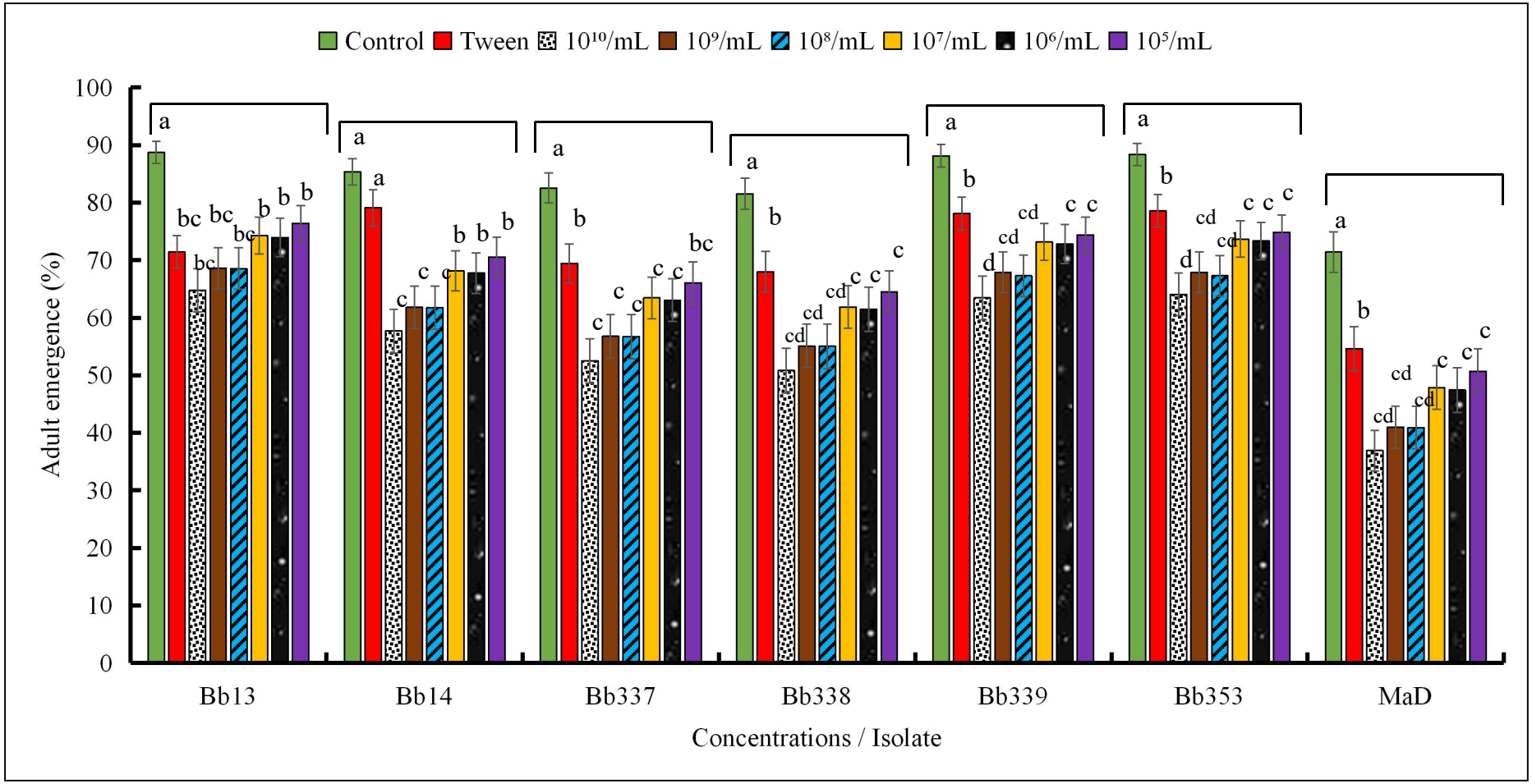


Figure 4.15. Adult emergence (%) of *Zeugodacus cucurbitae* in larval inoculation method treated with different concentrations of six isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and one isolate of *Metarhizium anisopliae* (MaD)

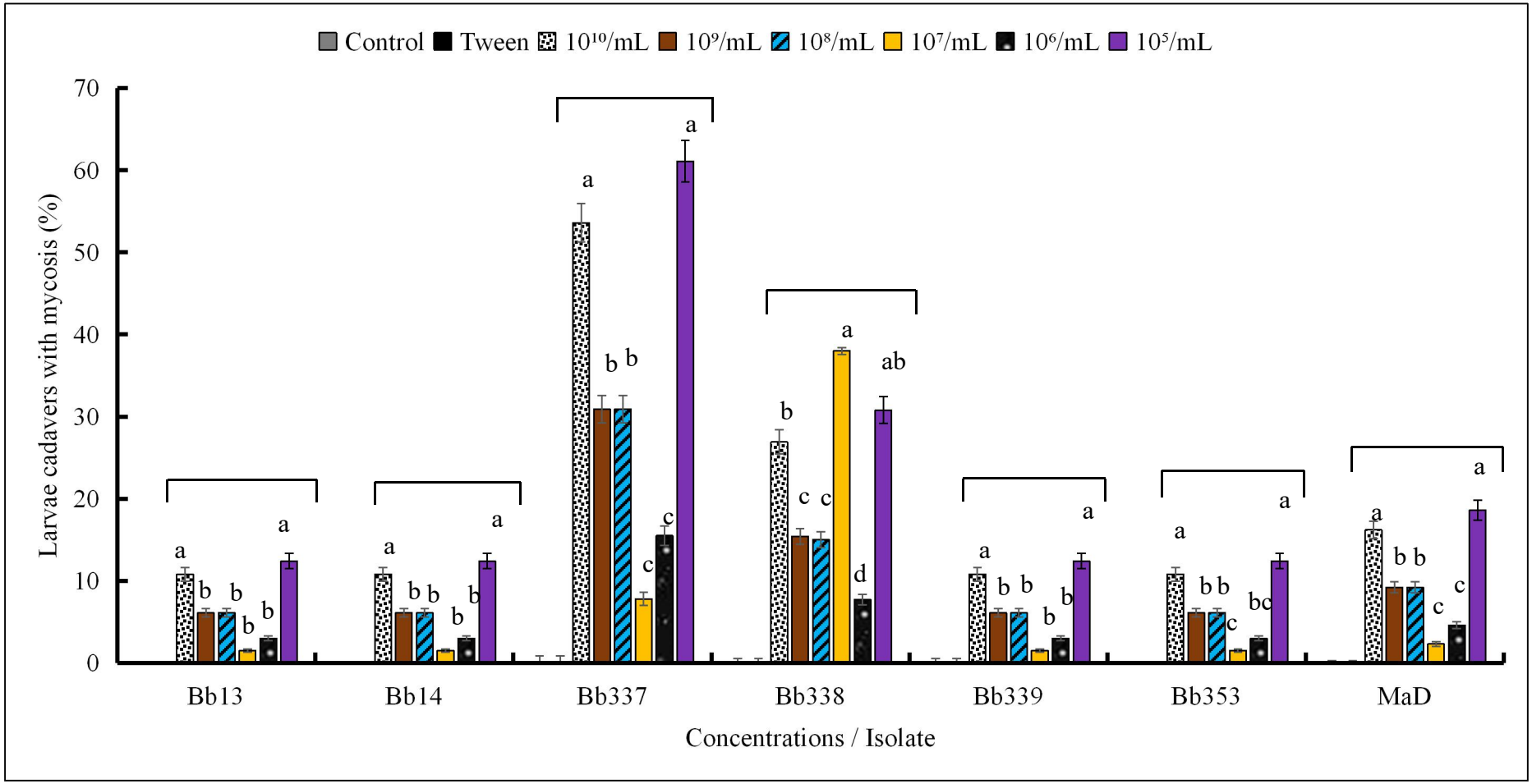


Figure 4.16. Cadavers (%) of *Zeugodacus cucurbitae* larvae with fungal growth after treatment different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under larval inoculation method

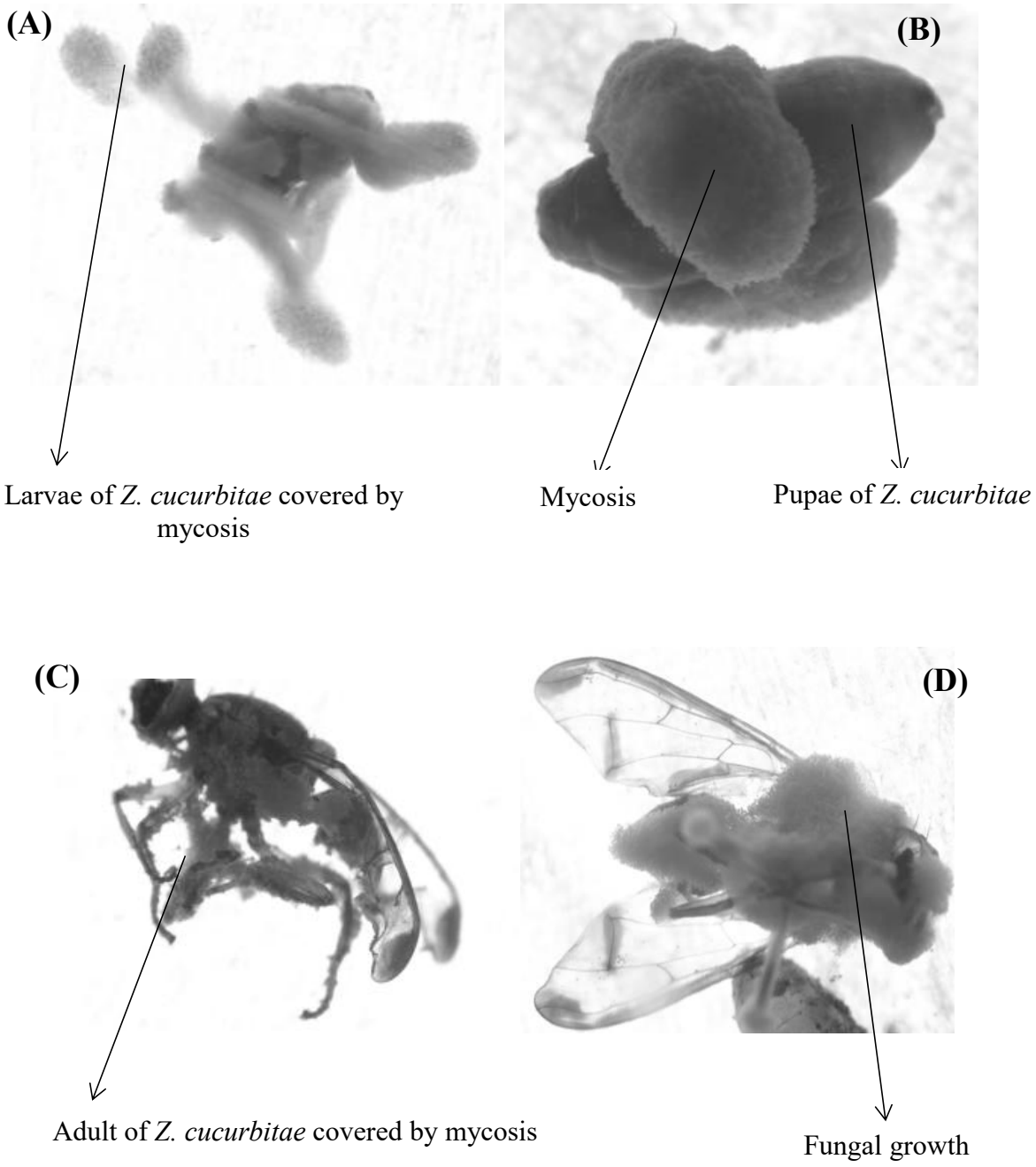


Plate 4.5. Cadavers of larvae, pupae and adults of *Zeugodacus cucurbitae* with fungal growth

The rates are 7.35 ± 2.49%; 9.44 ± 2.87%; 13.97 ± 3.52%; 14.99 ± 3.51%; 15.41 ± 3.81%; 16.99 ± 3.78% and 18.65 ± 3.96% for MaD; Bb14; Bb13; Bb337, Bb339, Bb338 and Bb353 respectively. The lowest percent pupae cadavers with mycosis was recorded when applying 10^5 conidia/mL per insect (Figure 4.17). However, significant differences were observed among concentrations ($P < 0.05$) with no differences among isolates.

Adults emergence rate from enclosed pupae showed highest percent adults cadavers with mycosis when applied at 10^{10} conidia/mL and 10^9 conidia/mL for all isolates. Bb338 ranked first with rates of 18.61 ± 4.88%, followed by Bb337 (16.43 ± 5.00%) and Bb14 (16.22 ± 4.536%) at a concentration of 10^{10} conidia/mL per insect. The lowest percent pupae cadavers with mycosis was recorded when applying 10^5 conidia/mL per insect (Figure 4.18). Adult's percent pupae cadavers with mycosis significantly differ among isolates ($P < 0.05$) and concentrations.

4.4.6 Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on pupae, adult mortality and under pupal inoculation method

All isolates showed high mortality rates when applied at any concentrations of conidia per insect with the lowest recorded with MaD (46.80 ± 2.46% to 50.75 ± 2.84%). However, all isolates caused more than 50% mortality rates at very low concentrations regardless the isolates tested (Figure 4.19). Pupae mortality rates were significantly different among isolates ($P < 0.05$) and concentrations ($P < 0.05$).

4.4.7 Pupae and adult cadavers of *Zeugodacus cucurbitae* with mycosis after infection with *Beauveria bassiana* and *Metarhizium anisopliae* under pupal inoculation method

Pupae cadavers inoculated with *MaD* showed the highest percent pupae cadavers with mycosis which ranged from 20.27 ± 3.73% (10^5 conidia/mL) to 32.42 ± 4.37% (10^8 conidia/mL). Treatments Bb337 and Bb338 shown percent pupae cadavers with mycosis varied from 11.87 ± 2.6% and 7.76 ± 1.93% (10^5 conidia/mL) to 20.27 ± 3.5% and 13.71 ± 2.81% (10^8 conidia/mL) respectively (Figure 4.20). There was a significant difference among isolates ($P < 0.05$) and concentrations ($P < 0.05$). Regarding percent adult cadavers with mycosis, high concentrations showed highest rates (37.93 ± 9.78% and 29.60 ± 5.39%) when applied at 10^9 conidia/mL respectively for Bb337 and Bb338. However, adults emerged from inoculated pupae and treated

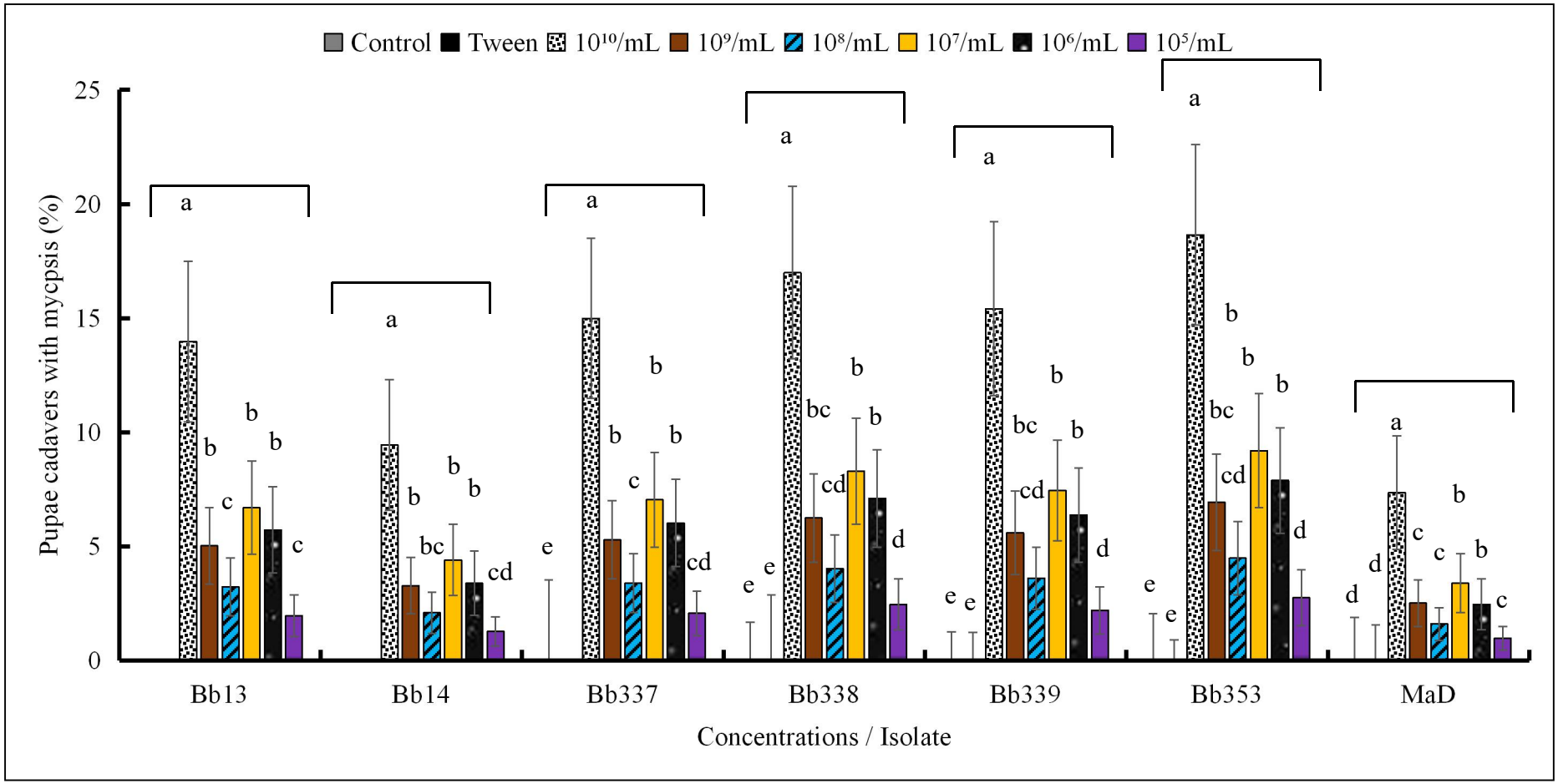


Figure 4.17. Cadavers (%) of *Zeugodacus cucurbitae* pupae with fungal growth after treatment different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under larval inoculation method

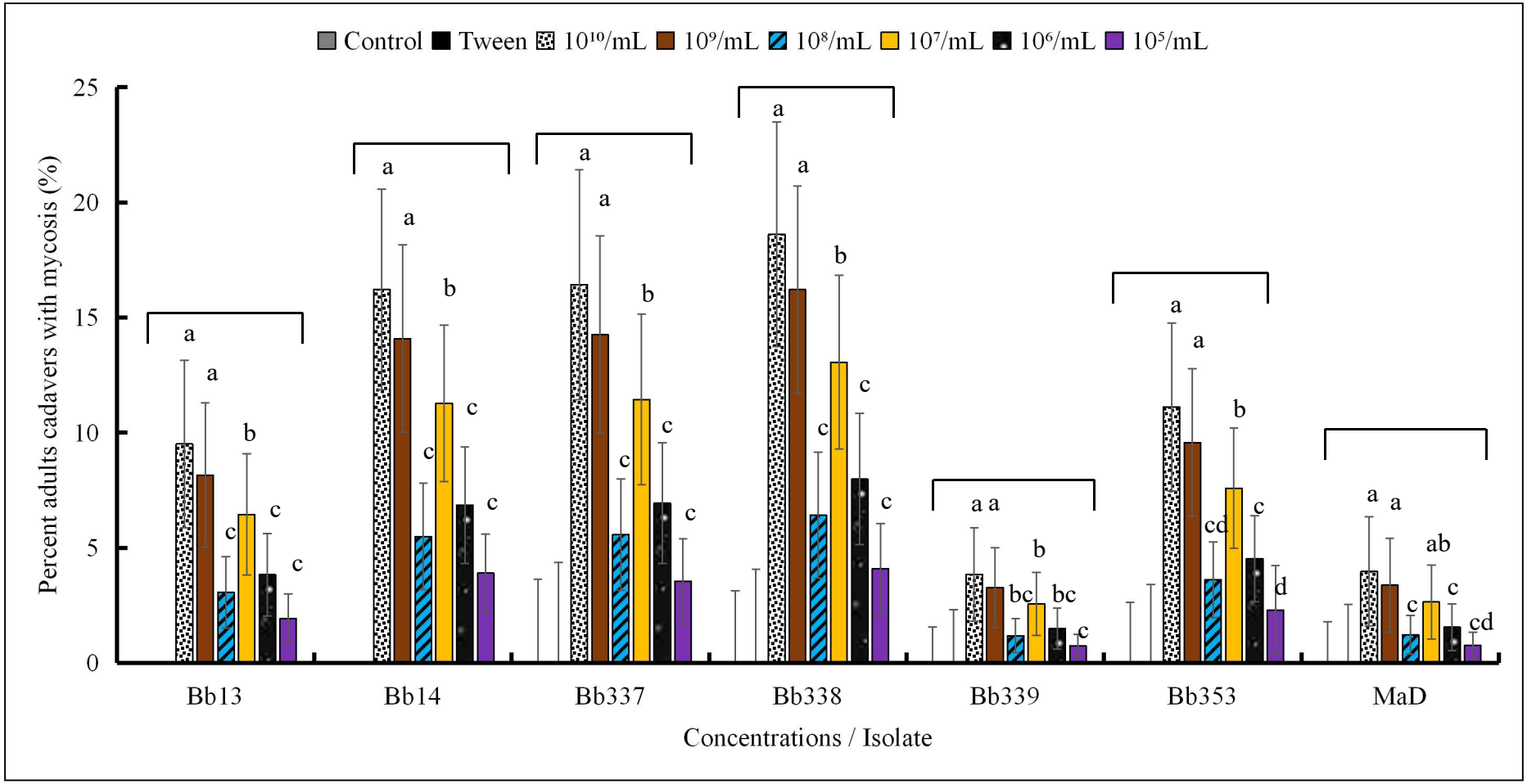


Figure 4.18. Cadavers (%) of *Zeugodacus cucurbitae* adults emerged from enclosed pupae with fungal growth after treatment different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under larval inoculation method

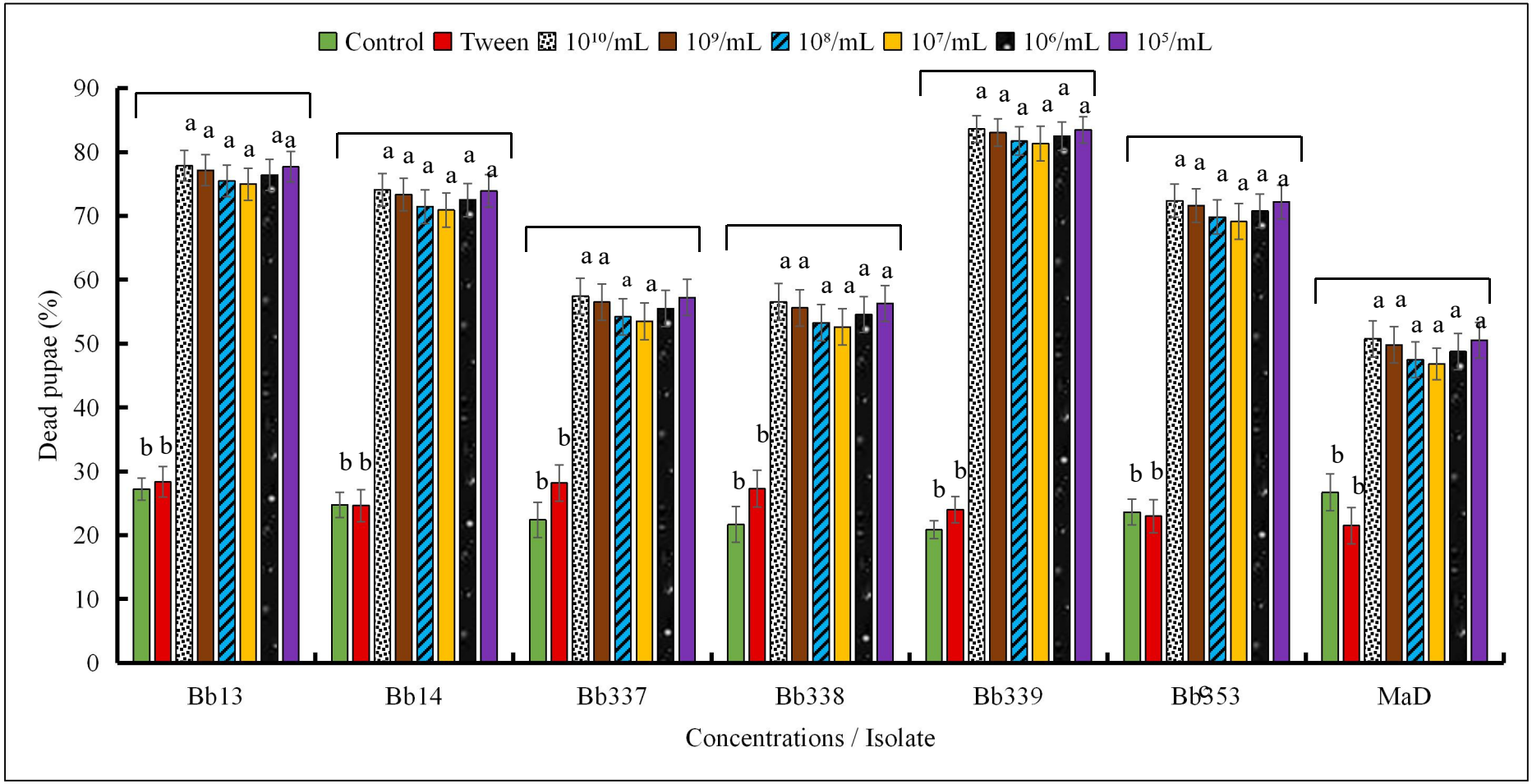


Figure 4.19. Mortality (%) of *Zeugodacus cucurbitae* pupae after treatment with different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under pupal inoculation method

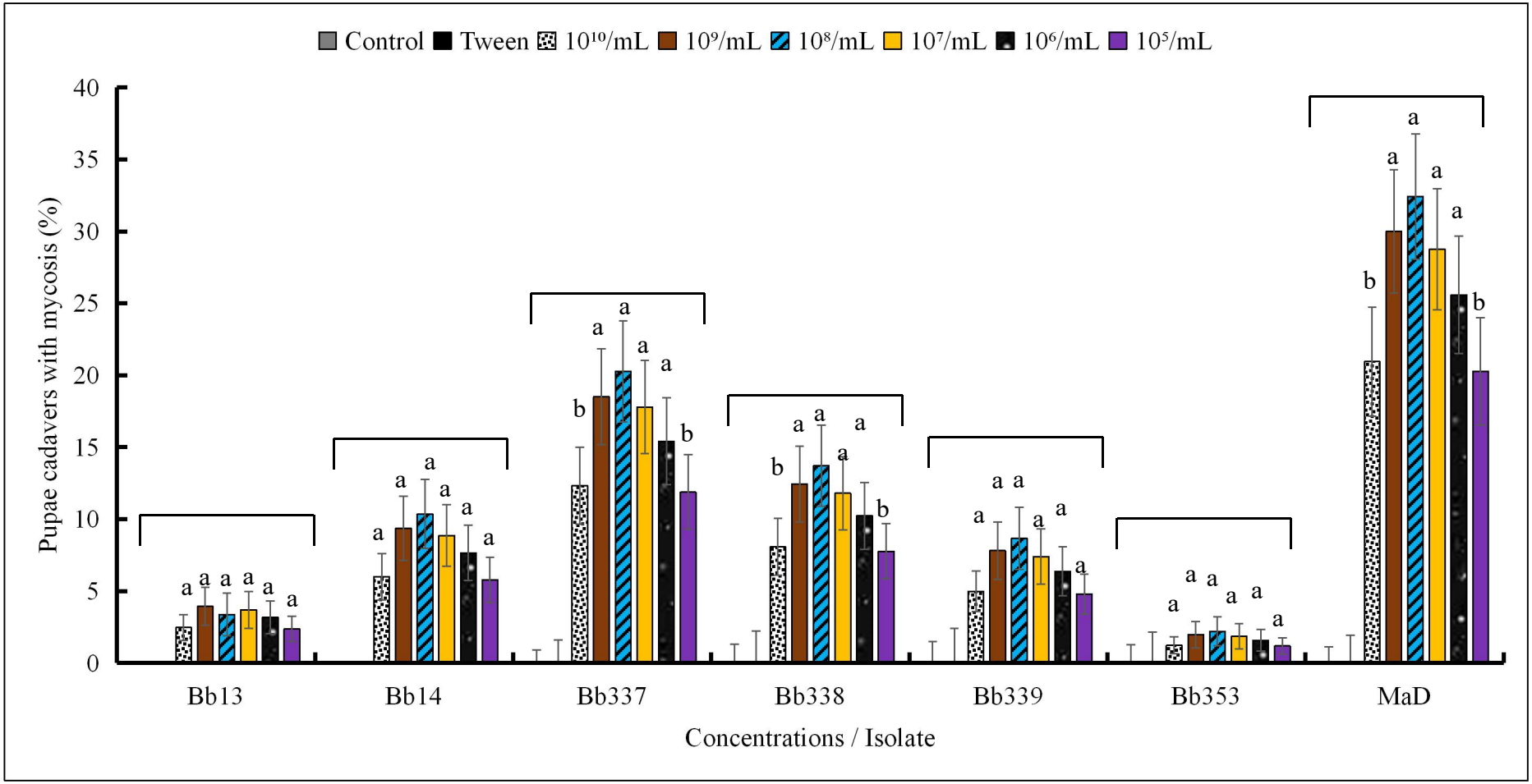


Figure 4.20. Cadavers (%) of *Zeugodacus cucurbitae* pupae with fungal growth after treatment with different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under pupal inoculation method

with treatments Bb339 and MaD did not show fungal growth (Figure 4.21). There were significantly different among isolates ($P < 0.05$) and concentrations ($P < 0.05$).

4.4.8 Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on adult mortality and dead adults with fungal growth under adults' inoculation method

When adults were directly inoculated with different isolates, highest percent adults cadavers with mycosis were recorded with Bb338 (36.00 ± 7.02%) followed Bb337 (28.84 ± 6.52%) when applied at 10^{10} conidia/mL (Figure 4.22). Significant differences were observed among isolates ($P < 0.05$) and concentrations ($P < 0.05$).

4.4.9 Effectiveness of *Beauveria bassiana* and *Metarhizium anisopliae* on the fecundity of fruit flies under adults inoculation method

The fecundity of *Z. vertebratus* was not significantly affected by fungal isolates. The highest number of pupae was recorded in the control 3.79 ± 1.36 pupae while the treatement with Tween 80 solution, gave 1.15 ± 0.46 pupae. At high concentrations of conidia per mL, the number of pupae was reduced as compared to adults infected with low concentrations of conidia of each isolate (Table 4.20). The number of pupae collected from fruits infested by adults inoculated with different isolates showed significant differences among concentrations ($P < 0.05$).

4.4.10 Lethal concentration value after treatment of the third instar larvae of *Zeugodacus cucurbitae* with various concentrations of *Beauveria bassiana* and *Metarhizium anisopliae*

The Cox regression analysis demonstrates that the different fungal doses used were significant indicators of *Zeugodacus cucurbitae* larval mortality ($P<0.05$). Isolates Bb337, Bb338 and Bb13 induced less than 50% mortality of the tested larvae. The death ratio curve decreased with an increase of fungal concentration (Figure 4.23). However, the death ratio increased with an increase in conidia suspensions concentration showing the existence of a dose-mortality response.

The required concentrations to kill 50% of third instar larvae of *Z. cucurbitae* ranged between 10^5 and 10^6 conidia/mL for isolate Bb13 while it fell between 10^6 and 10^7 conidia/mL for isolates Bb339, Bb353 and MaD (Figure 4.24).

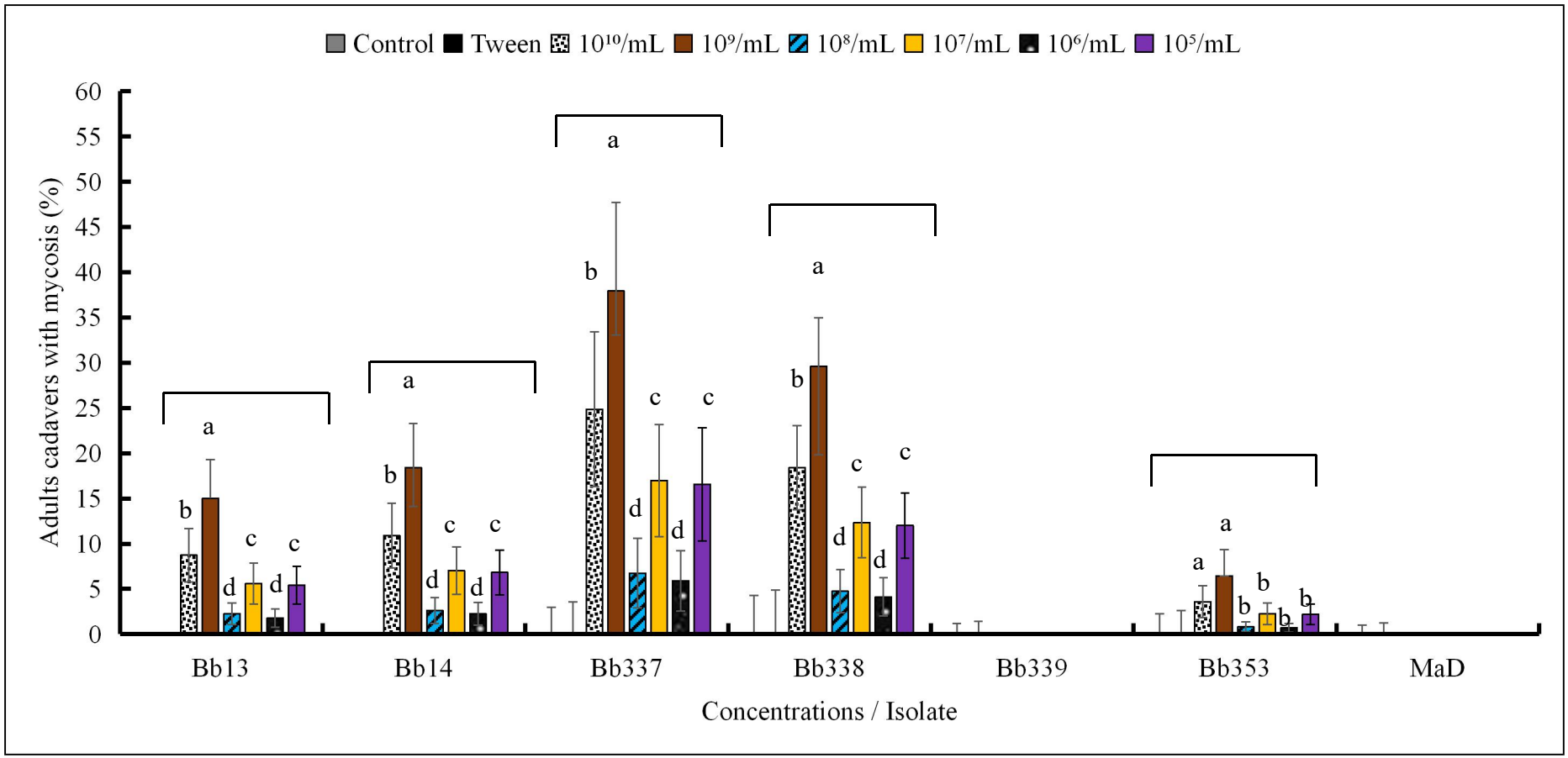


Figure 4.21. Cadavers (%) of *Zeugodacus cucurbitae* adults with fungal growth after treatment with different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under pupal inoculation method

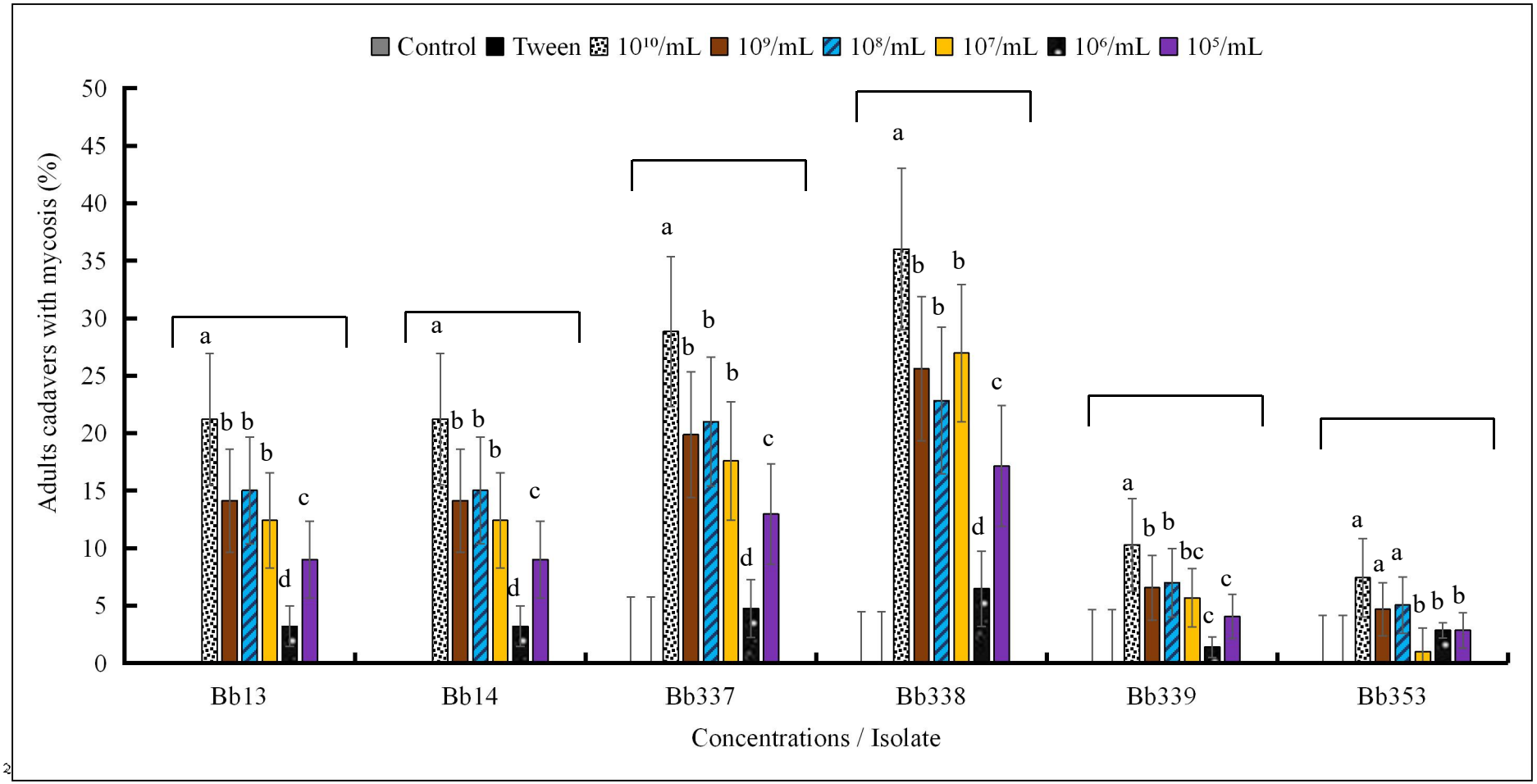


Figure 4.22. Cadavers of *Zeugodacus cucurbitae* adults with fungal growth after treatment with different concentrations of isolates of *Beauveria bassiana* (13, 14, 337, 338, 339, 353) and *Metarhizium anisopliae* (MaD) under adult inoculation method

Table 4.20. Number of collected pupae from adults infected with entomopathogenic fungi

Treatments	Isolate Bb13	Bb14	Bb337	Bb338	Bb339	Bb353
Control	2.64 ± 0.98b	3.79 ± 1.36b	2.17 ± 0.83b	2.13 ± 0.81b	3.74 ± 1.34b	1.98 ± 0.76b
Tween80	0.81 ± 0.33a	1.15 ± 0.46a	0.66 ± 0.28a	0.65 ± 0.27a	1.14 ± 0.45a	0.61 ± 0.27a
10^{10}conidia/mL	0.45 ± 0.20a	0.64 ± 0.28a	0.37 ± 0.17a	0.35 ± 0.16a	0.63 ± 0.27a	0.33 ± 0.15a
10^{9}conidia/mL	0.16 ± 0.09a	0.22 ± 0.13a	0.13 ± 0.08a	0.13 ± 0.07a	0.22 ± 0.12a	0.12 ± 0.07a
10^{8}conidia/mL	0.91 ± 0.37a	1.30 ± 0.51a	0.74 ± 0.31a	0.73 ± 0.30a	1.28 ± 0.50a	0.68 ± 0.28a
10^{7}conidia/mL	0.16 ± 0.09a	0.23 ± 0.13a	0.13 ± 0.08a	0.22 ± 0.13a	0.12 ± 0.07a	0.12 ± 0.07a
10^{6} conidia/mL	0.43 ± 0.19a	0.61 ± 0.26a	0.35 ± 0.16a	0.34 ± 0.15a	0.60 ± 0.26a	0.32 ± 0.15a
10^{5} conidia/mL	1.19 ± 0.47b	1.70 ± 0.65a	0.98 ± 0.39a	0.96 ± 0.39a	1.68 ± 0.64a	0.89 ± 0.36a

*Means within columns followed by the same letters are not significantly different at $\alpha = 0$.

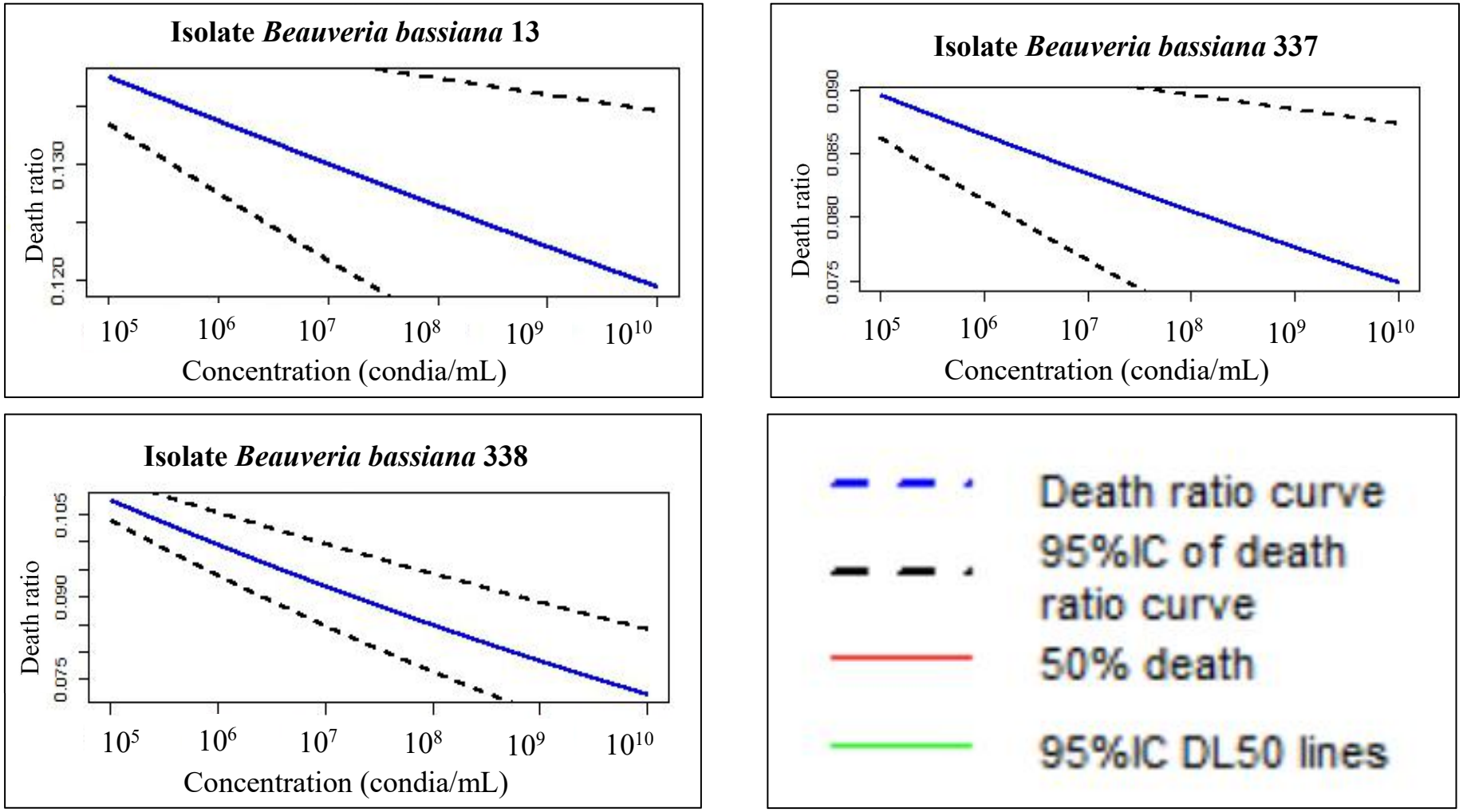


Figure 4.23. LC_{50} values after treatment of the larvae of *Zeugodacus cucurbitae* to various concentrations of Bb13, Bb337 and Bb338

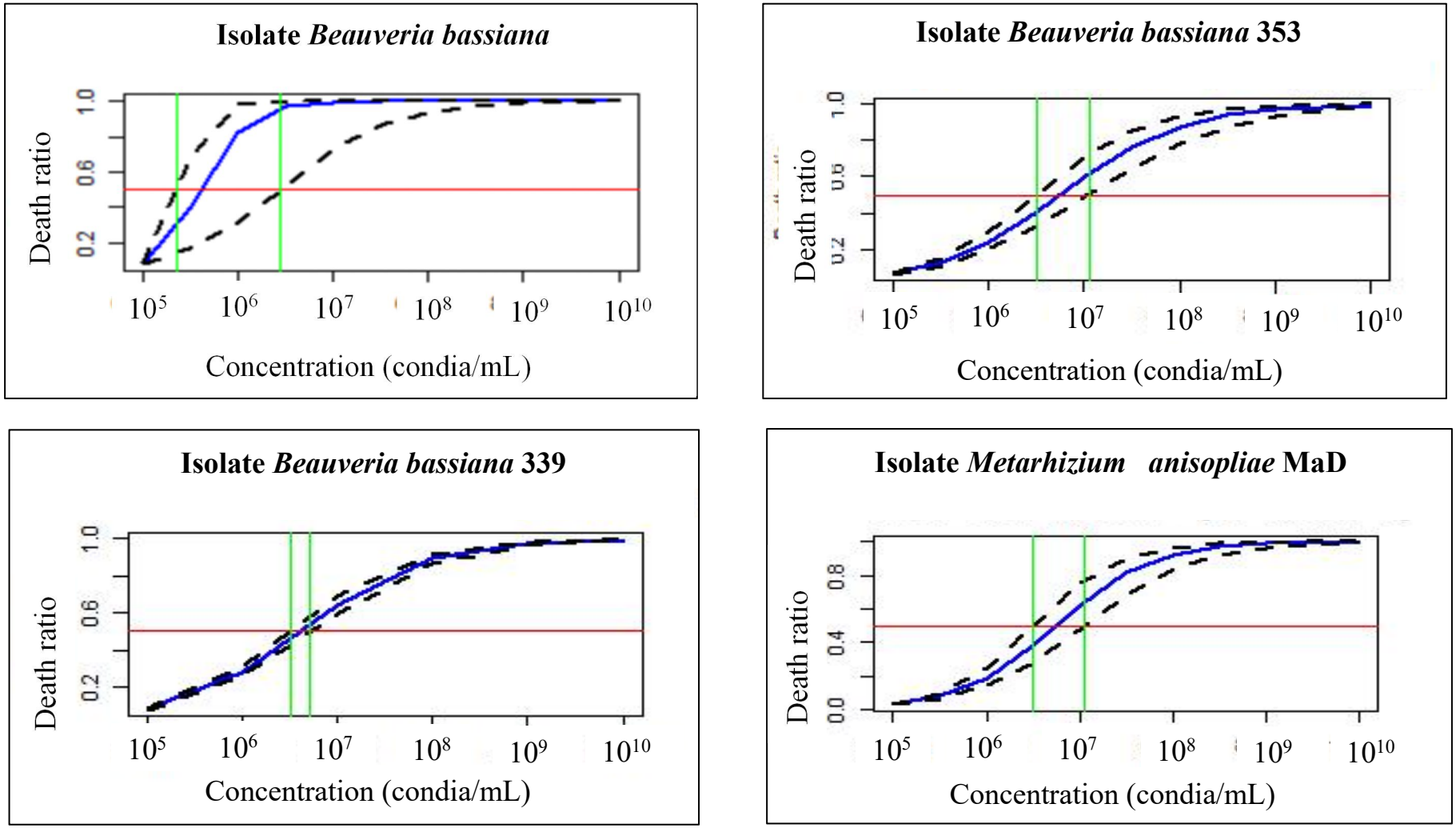


Figure 4.24. LC_{50} values after treatment of larvae of *Zeugodacus cucurbitae* to various concentrations of Bb14, Bb353, Bb339 and MaD

4.4.11 Effect of local fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on *Zeugodacus cucurbitae* and *Dacus vertebratus*

The effect of mixture one of local fungi (*A. niger, F. verticillioides, Botrytricum* sp. and an antibiotic, *Streptomycin* sp.) with either *B. bassiana* and *M. anisopliae* on mortality of *Z. cucurbitae* and *D. vertebratus* is presented in Table 4.21 and 4.22. Number of larvae cadavers of *D. vertebratus* with fungal growth of Bb338 (3.40 ± 0.24; 2.80 ± 1.49), Bb337 (2.40 ± 0.68; 2.20 ± 0.37) and MaD (1.60 ± 0.40; 2.40 ± 0.67) was higher than *A. niger* (1.60 ± 0.52; 1.33 ± 0.37) after treatment with the mixture of 50% and 100% of each isolate suspension respectively. Similarly, the highest number of larvae cadavers with mycosis was recorded with Bb337, Bb338 and MaD after mixed with *F. verticillioides, Botrytricum* sp. and an antibiotic, *Streptomycin* sp. However, number of cadaver larvae with *Botrytricum* sp. was higher than that of MaD.

Similar trend was observed in the number of cadaver larvae of *Z. cucurbitae* with mycosis. Isolates of *B. bassiana* and *M. anisopliae* recorded the highest number of cadavers with fungal growth. This showed that *Beauveria* and *Metarhizium* took over local isolates and can easily be spread.

4.4.12 Genotoxicity effect of fungal suspensions through *Allium cepa* method

Effect of different concentrations of isolates on roots growth significantly varied among applied concentrations (25%, 50%, 100%, 200% and 300%) (Table 4.23). The maximum root growth was observed in the controls (30.60 ± 8.00 roots) and there were no morphological deformities found. However, at tested concentrations, the highest root growth was obtained from solution made with 25% of the recommended concentrations (27.40 ± 7.21 for Bb337 and 21.00 ± 5.61 for Bb338). The number of roots decreased with an increase in the concentrations for each isolate. Evidently, at great concentrations, the treatments cause a delay in the growth of *A. cepa*. The number roots were significantly different among concentrations ($P < 0.05$).

In terms of roots, the longest root length was observed when treated with 50% of the recommended concentration of Bb337 (2.18 ± 0.11 cm) and Bb338 (2.08 ± 0.12 cm). The shortest roots length was obtained at high concentrations of Bb337 (0.44 ± 0.16) and Bb338 (0.37 ± 0.20 cm) (Table 4.24). Roots length were significantly different among concentrations ($P < 0.05$).

Table 4.21. Number of larvae cadavers of *Dacus vertebratus* with fungal growth after inoculation with mixture of local isolates, *Beauveria bassiana* and *Metarhizium anisopliae*

	Aspergillus niger	*B. bassiana* 337	*B. bassiana* 338	*M. anisopliae* D
50%	1.60 ± 0.52c	2.40 ± 0.68a	3.40 ± 0.24b	1.60 ± 0.40c
100%	1.33 ± 0.37b	2.20 ± 0.37a	2.80 ± 1.49a	2.40 ± 0.67a
	Fusarium sp.	*B. bassiana* 337	*B. bassiana* 338	*M. anisopliae* D
50%	0.67 ± 0.28c	2.00 ± 0.55a	1.80 ± 0.37a	1.40 ± 0.51b
100%	1.20 ± 0.30b	1.60 ± 0.44b	2.40 ± 0.75a	1.60 ± 0.40b
	Botryotricum sp.	*B. bassiana* 337	*B. bassiana* 338	*M. anisopliae* D
50%	1.40 ± 0.99b	1.60 ± 1.52b	3.20 ± 1.64a	1.40 ± 1.34b
100%	1.53 ± 1.13b	2.20 ± 0.84a	2.40 ± 1.14a	1.20 ± 1.30b
	Streptomycin sp.	*B. bassiana* 337	*B. bassiana* 338	*M. anisopliae* D
50%	0.80 ± 0.35b	1.40 ± 0.51a	1.40 ± 0.40a	0.80 ± 0.20b
100%	0.80 ± 0.35c	2.00 ± 0.32a	1.60 ± 0.68b	1.40 ± 0.24b

*Means within rows followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.22. Number of pupae cadavers of *Zeugodacus cucurbitae* with fungal growth after inoculation with mixture of local isolates, *Beauveria bassiana* and *Metarhizium anisopliae*

	Aspergillus niger	*B. bassiana 337*	*B. bassiana 338*	*M. anisopliae D*
50%	1.67 ± 0.37c	2.60 ± 0.40b	3.40 ± 0.24a	2.40 ± 0.51b
100%	1.40 ± 0.60b	1.60 ± 0.24b	1.80 ± 0.49a	1.60 ± 0.43b
	Fusarium sp.	*B. bassiana 337*	*B. bassiana 338*	*M. anisopliae D*
50%	1.27 ± 0.36b	2.80 ± 0.58a	2.80 ± 0.58a	2.60 ± 0.51a
100%	0.60 ± 0.23b	1.60 ± 051a	1.80 ± 0.58a	1.80 ± 0.49a
	Botryotricum sp.	*B. bassiana 337*	*B. bassiana 338*	*M. anisopliae D*
50%	0.20 ± 0.41c	2.20 ± 1.92b	2.60 ± 1.34b	3.40 ± 1.52a
100%	0.60 ± 0.63c	0.80 ± 0.84b	2.00 ± 1.58a	1.20 ± 1.30b
	Streptomycin sp.	*B. bassiana 337*	*B. bassiana 338*	*M. anisopliae D*
50%	0.60 ± 0.29c	2.80 ± 0.58a	3.00 ± 0.55a	2.60 ± 0.51b
100%	0.73 ± 0.20b	1.40 ± 0.40a	1.60 ± 0.24a	1.20 ± 0.37b

*Means within rows followed by the same letters are not significantly different at α = 0.05

Table 4.23. Number and roots length of *Allium cepa*

Concentrations (%)	*Beauveria bassiana 337*		*Beauveria bassiana 338*	
	Number of Root (cm)	Roots Length (cm)	Number of Root (cm)	Roots Length (cm)
25%	27.40 ± 7.21c	2.12 ± 0.12c	21.00 ± 5.61c	1.89 ± 0.13b
50%	18.20 ± 4.91b	2.18 ± 0.11c	15.80 ± 4.31b	2.08 ± 0.12b
100%	17.20 ± 4.66b	0.89 ± 0.12b	14.40 ± 3.96b	1.38 ± 0.13b
200%	14.00 ± 3.86a	0.44 ± 0.13a	11.60 ± 3.26b	1.80 ± 0.14b
300%	8.60 ± 2.51a	0.44 ± 0.16a	5.80 ± 1.80a	0.37 ± 0.20a
Distilled water	30.60 ± 8.00c	1.70 ± 0.09bc	30.60 ± 8.00c	1.70 ± 0.09b
Tween80	30.60 ± 8.00c	2.07 ± 0.09bc	30.60 ± 8.00c	2.07 ± 0.09b
K-Optimal	0.00 ± 0.00d	0.00 ± 0.48a	0.00 ± 0.00d	0.00 ± 0.48a

*Means within columns followed by the same letters are not significantly different at $\alpha = 0.05$

Table 4.24. Effects of *Beauveria bassiana* concentrations on the sharp and forms of *Allium cepa* L. roots

Concentrations (%)	*Beauveria bassiana* 337			*Beauveria bassiana* 338		
	Roots Forms			Roots Forms		
	Vertical	Oblique	Returns	Vertical	Oblique	Returns
25%	45.98 ± 4.26	54.01 ± 4.26	0.00 ± 0.00	34.29 ± 4.63	57.14 ± 4.83	8.57 ± 2.73
50%	3.30 ± 1.87	95.6 ± 2.14	1.10 ± 1.10	39.51 ± 5.43	39.51 ± 6.16	20.99 ± 4.52
100%	5.81 ± 2.52	94.19 ± 2.52	0.00 ± 0.00	2.78 ± 1.94	94.44 ± 2.70	2.78 ± 1.94
200%	45.71 ± 5.95	54.29 ± 5.95	0.00 ± 0.00	20.69 ± 5.32	67.24 ± 9.16	12.07 ± 4.28
300%	2.33 ± 2.30	97.67 ± 2.30	0.00 ± 0.00	37.93 ± 9.01	58.62 ± 9.15	3.45 ± 3.39
Distilled water	3.27 ± 1.43	94.12 ± 1.90	2.61 ± 1.29			
Tween80	2.61 ± 1.29	96.08 ± 1.57	1.31 ± 0.92			

*Means within columns followed by the same letters are not significantly different at $\alpha = 0.05$

The roots were whitish in colour, unbroken straight aquatic and lives. There were vertical or oblique in forms. Different treatment has no toxic effect on the growth of onions. Roots were stretched down. Comparatively, the chemical pesticide was toxic and onions failed to grow and the substance caused a change in colour of the bulb (Plate 4.6 and 4.7).

4.4.13 Bio-efficacy of entomopathogenic fungi in the control of fruit flies' species infesting *Citrullus lanatus*

The different treatments of entomopathogenic fungi (Bb337 and Bb338; MaD, Ma ICIPE-69 have been found to be effective against fruit flies infesting *C. lanatus* under field condition. Occurrence and abundance of spiders varied significantly among treatments during rainy season ($P < 0.05$). The number of spiders was highest in treatment Bb338 (24.21 ± 0.06) and (11.79 ± 0.04) during dry and rainy seasons. The lowest number of spiders were recorded with treatments ICIPE-69 (6.72 ± 0.03) and (3.28 ± 0.02) at both seasons. No spiders were recorded on plots treated with the chemical insecticide, K-Optimal (Table 4.24). Less spiders were however present during rainy season as compared to their abundance during dry season.

4.4.14 Effect of fungal application on some beneficial organisms

The black garden ants (*Lasius niger*) have been found preying on third instar larvae of fruit flies after they have jumped from the fruit to pupate in the soil (Plate 4.8). By so doing, ants reduced fruit flies population in the field. However, occurrence and abundance of flies varied significantly among treatments ($P < 0.05$). The probability of having no ants present was 37.43% in plots treated with the chemical pesticide k-optimal as compared to the control and other treatments. Five ants' nests were found in over 70% of treated plots (an average of 3 plots).

Weekly fruits samples showed reduction in the mean number of pupae after incubation. The highest number of pupae was in untreated plots (control) as compared to others treatments. The number of pupae decreased over the week in all fruits samples collected from treated plots while the number increased in the control. All treatments were effectively reduced fruit flies population in terms of number of pupae produced weekly (Figure 4.25 and 4.26). However, among treatments, there was no significant difference.

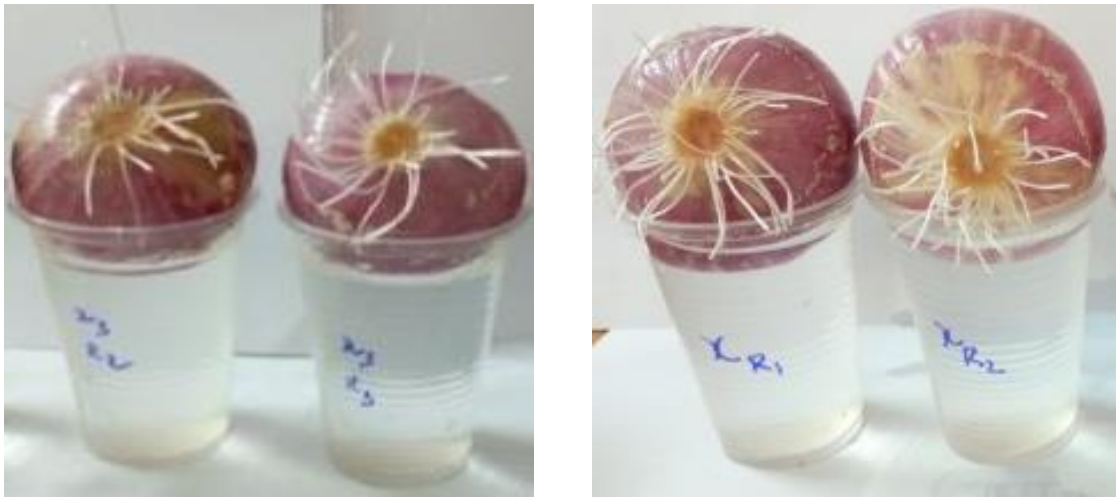


Plate 4.6a. Samples of onion bulbs showing the rootlets already grown after applying different concentration of the treatment *Beauveria bassiana* 337

Plate 4.6b. Samples of onion bulbs showing the rootlets already grown after applying different concentration of the treatment *Beauveria bassia*na 338

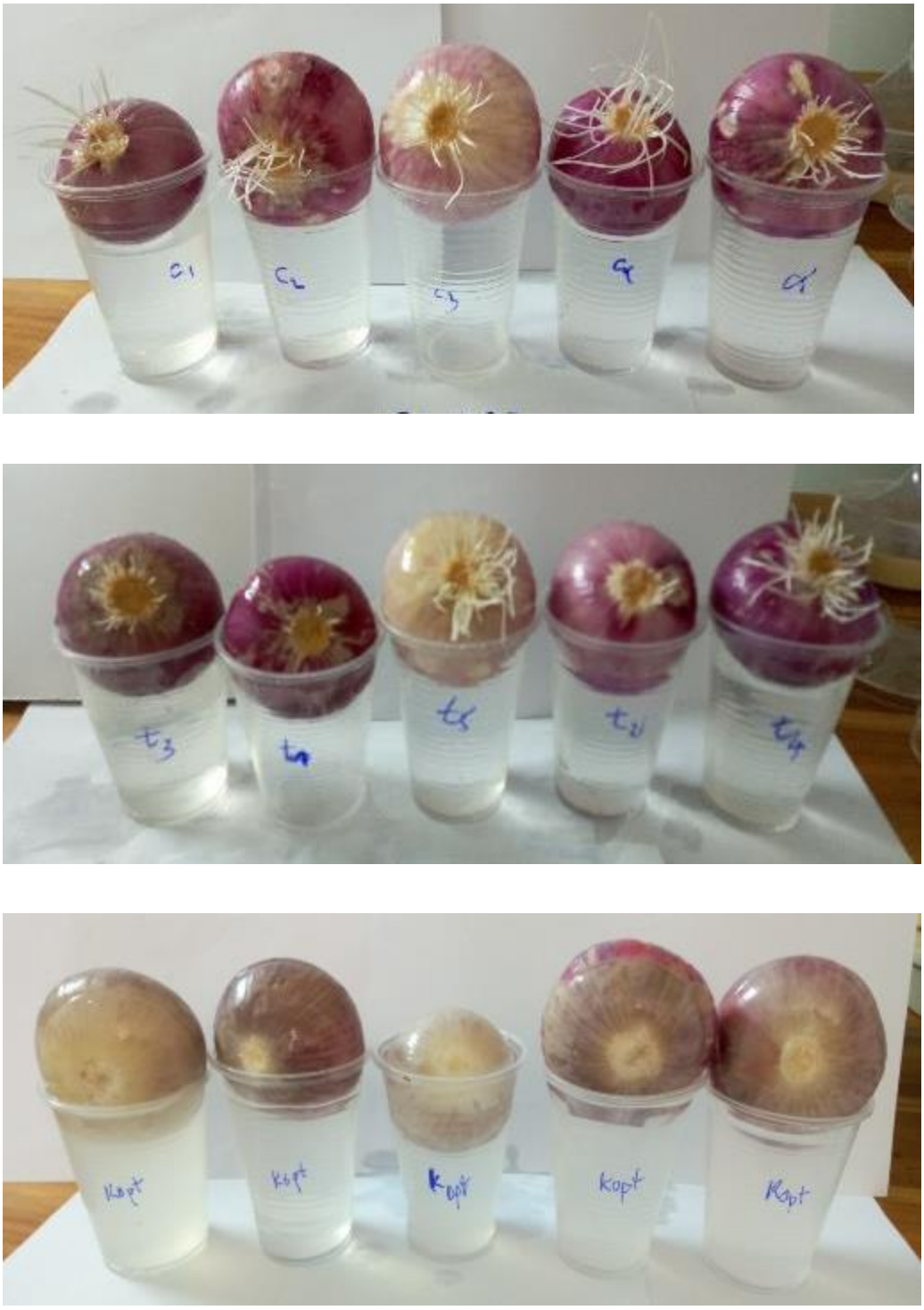


Plate 4.7. Samples of onion bulbs showing the rootlets already grown after applying the chemical insecticide, K-Optimal and different controls

a = Control 1 = Distilled water

b = Control 2 = Tween80

c = Chemical pesticide, K-Optimal

Table 4.24. Number of spiders in treated plots

	Dry season	Rainy season
Bb337	14.79 ± 0.05a	7.21 ± 0.03b
Bb338	24.21 ± 0.06b	11.79 ± 0.04b
Control	21.52 ± 0.06b	10.48 ± 0.03b
ICIPE-69	6.72 ± 0.03a	3.28 ± 0.02a
MaD	10.76 ± 0.04a	5.24 ± 0.02a
K-Optimal	-	0.00 ± 0.00a

*Means within columns followed by the same letters are not significantly different at $\alpha = 0.05$

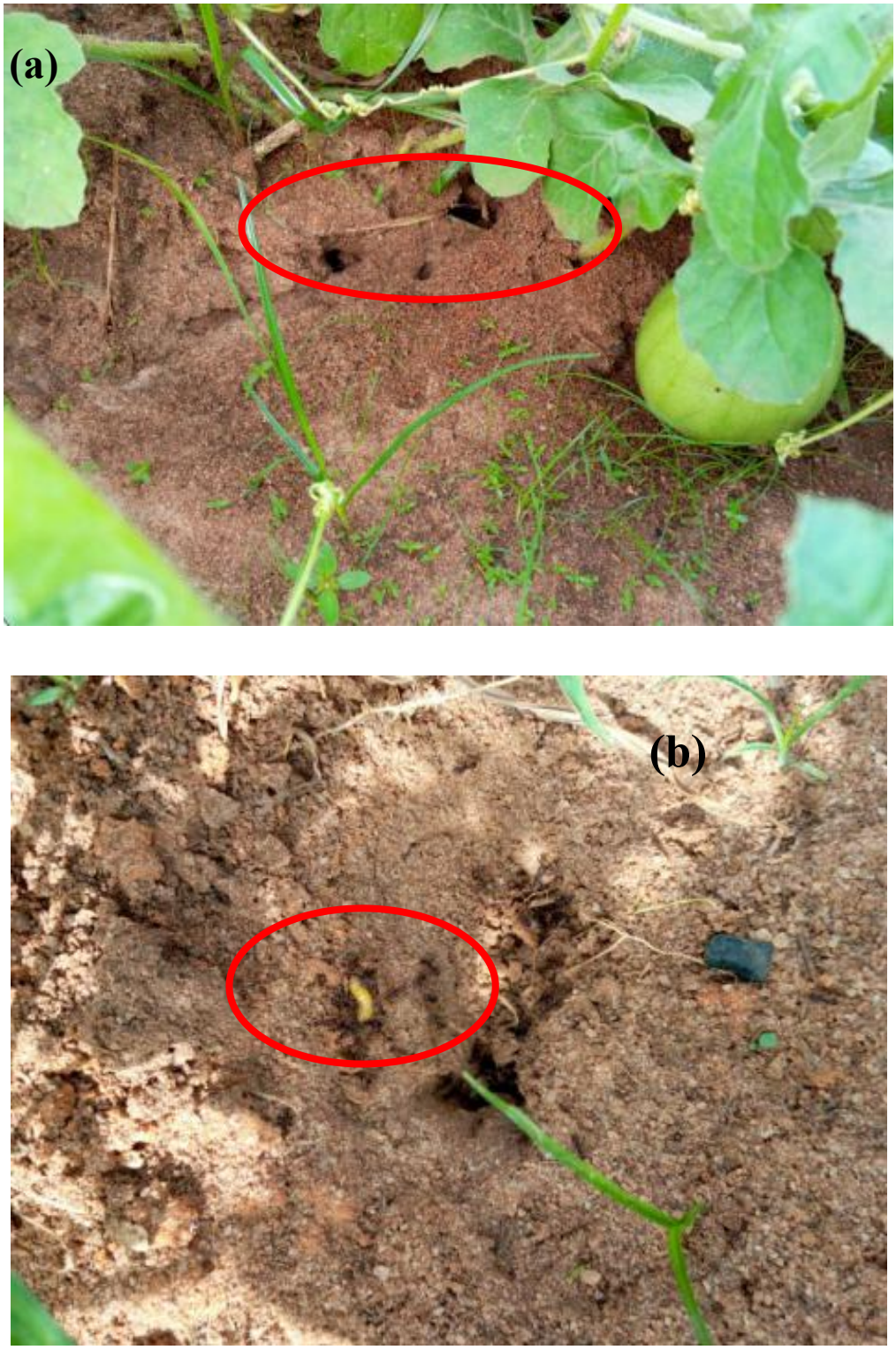


Plate 4.8 Presence of a) ants' nest and b) black garden ant, *Lasius niger* preying on larvae of fruit fly in the treated plot

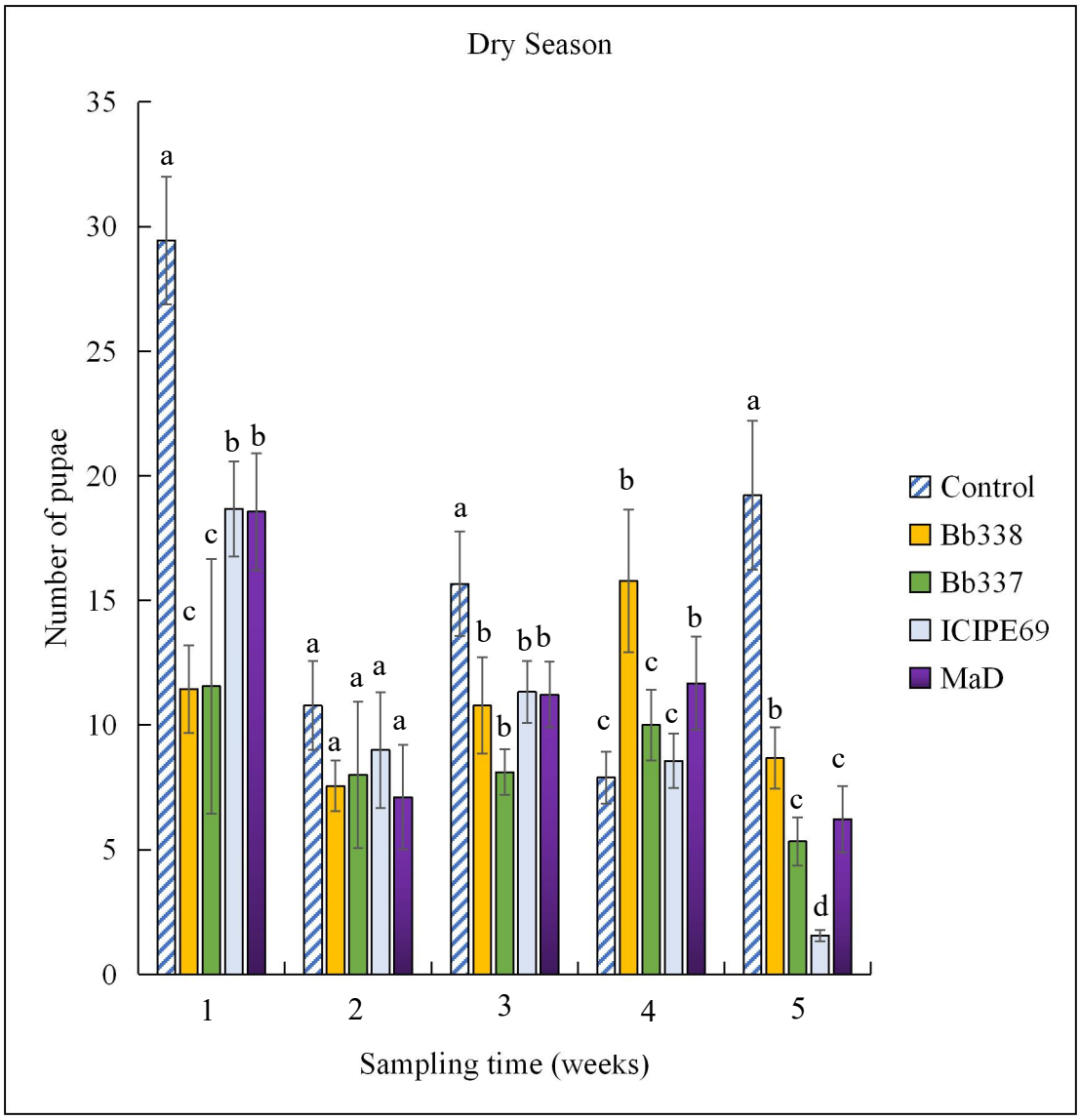


Figure 4.25. Number of pupae collected from fruits samples during dry season

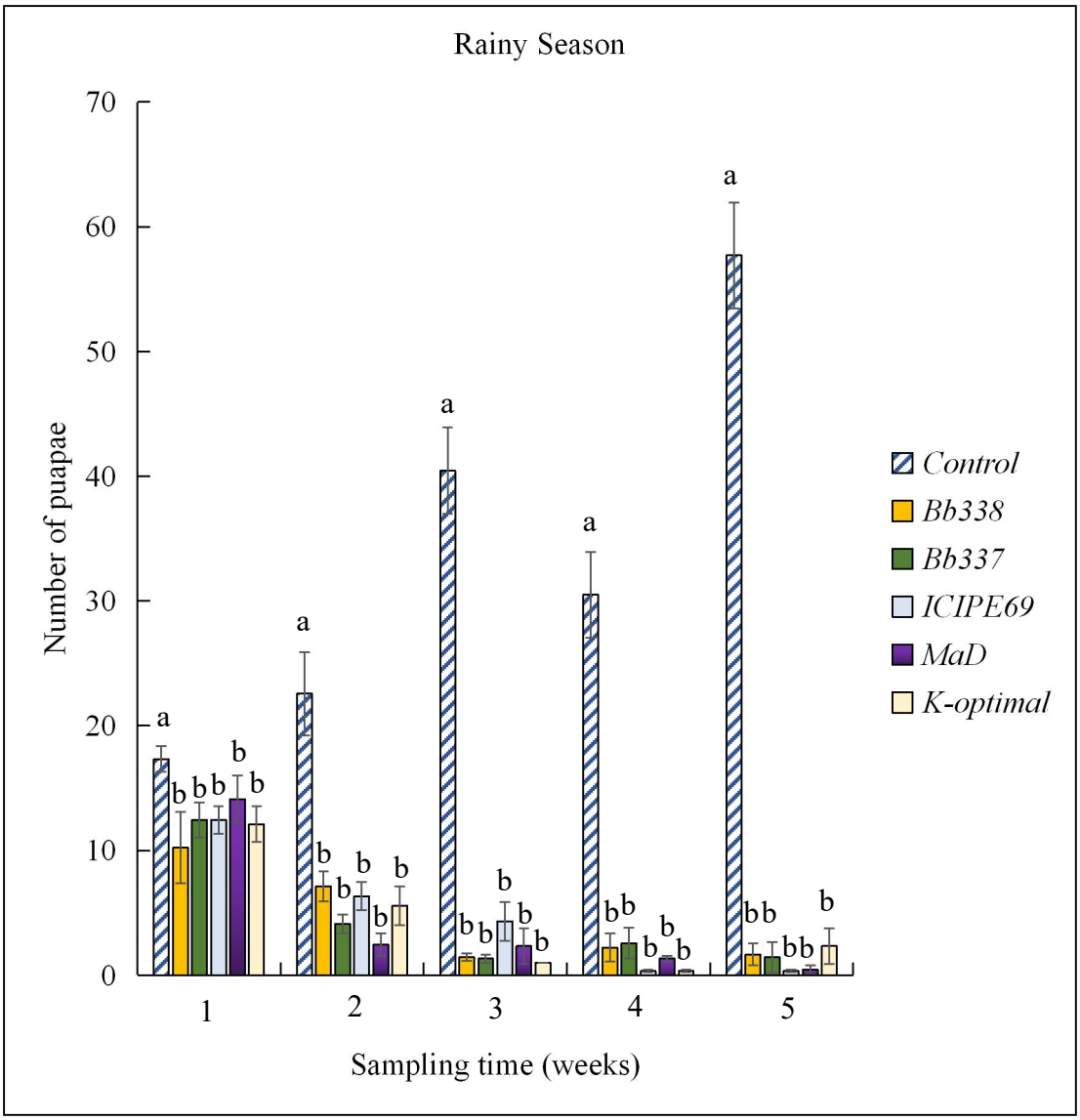


Figure 4.26. Number of pupae collected from fruits samples during rainy season

4.4.15 Fruit fly occurrence and infestation after treatment with isolates of entomopathogenic fungi

Dacus vertebratus was detected across seasons. During dry season, infestation densities ranged from 11.98 to 57.97 pupae per kg of fruits from treated plots with each treatment having different levels of infestation. The highest infestation rate (63.62 pupae/kg) was recorded in the control, followed by plots treated with Bb338 (57.71 pupae/kg), ICIPE-69 (51.25 pupae/kg), Bb337 (40.02 pupae/kg) and MaD (11.88 pupae/kg). However, infestation rate was not significantly different among treatments. The tendency of infestation load (number of pupae per kilogram of fruit) significantly varied during rainy season. Plots treated with ICIPE-69 had the highest fruit fly infestation per kilogram (120.20 pupae/kg), followed by treatment Bb338 (48.61% pupae/kg) and K-Optimal (48.41% pupae/kg) and lastly plots treated with Bb337 (44.48% pupae/kg) (Figure 4.27). There was no significant difference in the infestation rate by fruit flies among treatments.

4.4.16 Fruit yield as affected by isolates of entomopathogenic fungi

During dry season, fruit were more infested by fruits flies and other biotic and abiotic factors. Treatment ICIPE-69 had less damaged fruits (1.25 tons/ha) with the highest harvested fruit damaged caused by other factors (2.74 tons/ha). However, the highest fruits yield was recorded with the treatment Bb337 (4.36 tons/ha) followed by treatment ICIPE-69 (4.17 tons/ha) and MaD (3.96 tons/ha) (Table 4.26, Plate 4.9). the yield of fruits damaged by fruits flies was not significantly different among treatments.

The yield of harvested fruits was higher during rainy season. The treatment *MaD* had high undamaged fruits (148.75 tons/ha) as compared to the yield recorded when treated with the chemical pesticide K-Optimal (145.83 tons/ha). Application of Bb337 also gave low undamaged fruits production was 136.67 tons/ha followed by Bb338 (119.17 tons/ha). There was no significant difference in the yield of fruits damaged by fruits flies.

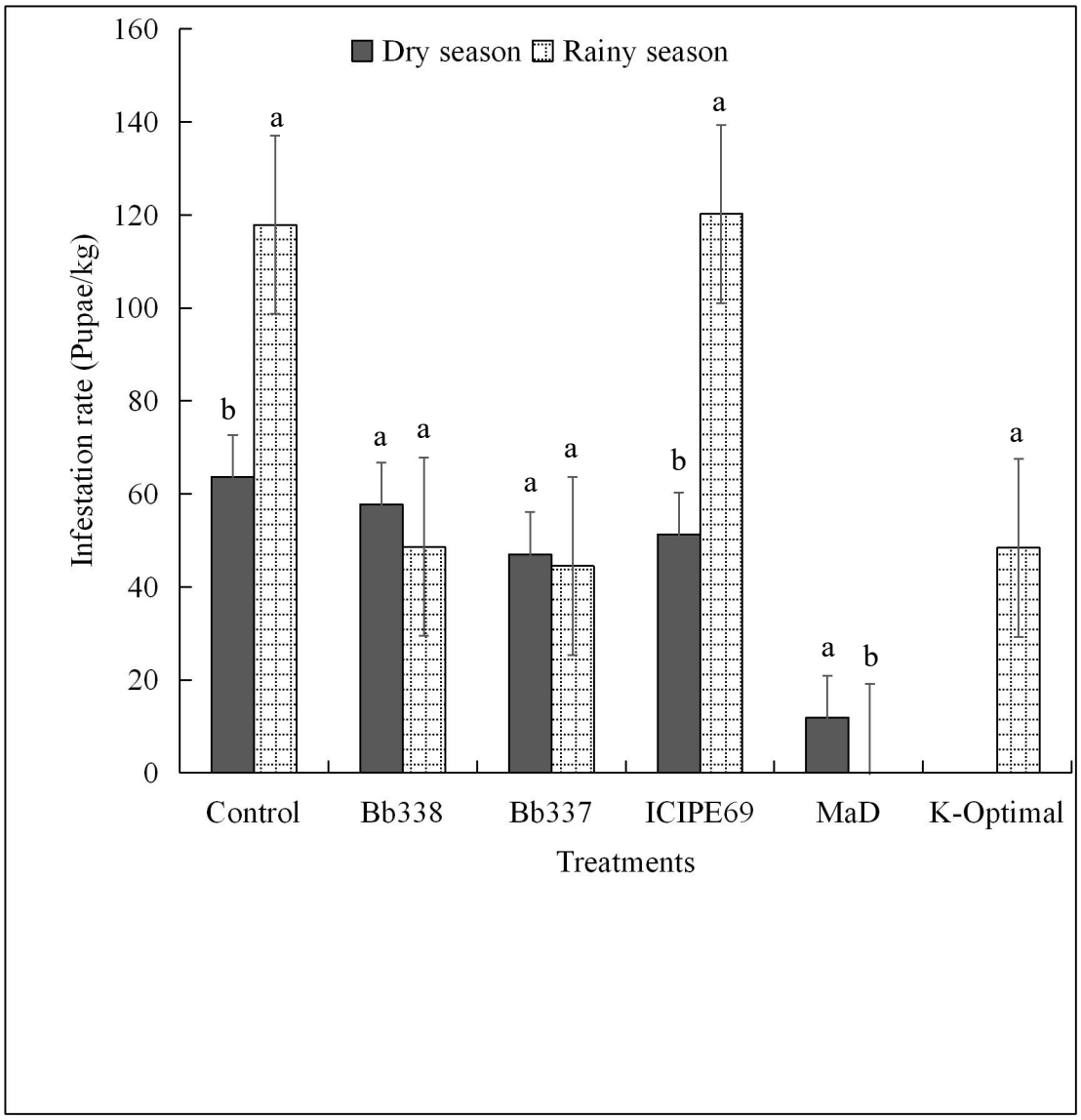


Figure 4.27. Variation in infestation per kilogramme of watermelon fruit collected from different seasons

Plate 4.9 Damaged fruits due to other biotic and abiotic factors

Table 4.25. Yield (in tons per ha) of *Citrullus lanatus* (watermelon) under different treatment conditions in the farmer's fields during rainy season

	Damaged by fruits flies		Damaged by others factors		Damaged		Undamaged fruits	
	Dry season	Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season	Rainy season
Control	6.67 ± 1.17a	10.83 ± 3.21a	0.00 ± 0.00c	5.83 ± 2.98a	6.67 ± 2.93a	1.67 ± 0.94c	5.42 ± 2.13a	90.42 ± 12.78a
Bb338	2.29 ± 0.96b	5.00 ± 1.83c	1.46 ± 0.87b	3.33 ± 1.99b	3.75 ± 2.13b	1.46 ± 0.88c	1.86 ± 074b	119.17 ± 15.90a
Bb337	3.54 ± 1.02b	7.92 ± 2.87b	2.71 ± 1.03a	6.67 ± 3.01a	6.46 ± 2.94a	2.29 ± 1.23c	4.38 ± 2.46a	136.67 ± 23.21a
ICIPE69	1.25 ± 0.27c	8.13 ± 2.77b	2.74 ± 1.04a	5.63 ± 2.76a	2.50 ± 1.78b	6.25 ± 3.12b	4.17 ± 2.34a	115.83 ± 21.34a
MaD	3.75 ± 1.09b	0.00 ± 0.00d	2.57 ± 2.34a	5.63 ± 2.65a	6.46 ± 3.10a	8.33 ± 3.62a	3.96 ± 2.84a	148.75 ± 25.23a
K-Optimal		4.58 ± 2.31c		3.55 ± 1.83b		8.13 ± 3.24a		145.833 ± 23.73a

*Means within columns followed by the same letters are not significantly different at $\alpha = 0.05$ (Kruskal test)

CHAPTER FIVE

DISCUSSION

There were significant variations in the effect of vegetables host fruit, parental ages and temperature on some basics biological aspects of *D. vertebratus* and *Z. cucurbitae* in the laboratory. The ability of the flies to complete their development on a particular host is an advantage for a successful adaptability. However, the quality of the host determined major differences in the life cycle of fruit flies. Another factor that may affect a suitable condition for the development of fruit fly is the temperature which affects immature development of fruits flies (Ekesi *et al.,* 2016). In the present experiment, the total development time was affected by the vegetable host fruit but not by parental age and temperature. However, the total developmental time of *D. vertebratus* increased when the temperature decreased. This is in agreement with works done by Mkiga and Mwatawala, (2015) in which an augmentaion of the temperature caused a reduction of the duration of total development of *Z. vertebratus.* Duration of host instars of *Z. cucurbitae* varied conversely with temperature. Similar trend was reported in *Bactrocera dorsalis* (Vargas *et al.,* 2000) and in *Ceratitis capitata* (Duyck *et al.,* 2004). Total developmental time (19 days) of *D. vertebratus* obtained at 25 ± 1°C in cucumber is more or less longer than the duration reported by Mkiga and Mwatawala, (2015) (17.2 days) and an average of 17.80 days by Huang and Chi, (2012) in the same vegetable host at the same temperature with *Z. cucurbitae*. The shortest development time was observed on *magda* (16 days).

Watermelon known as the preferred host of *D. vertebratus* was less infested in this study. This could have been due to the level of maturity stage, weight, juice and the mould that covered their surface. This may have drowned the immature stages of the larvae. Results are a little bit in contradiction with some findings that shown watermelon to be highly infested by *D. vertebratus* in the field (White, 2006). This may be due to the fact that we used part of mature fruits in our experiment which favoured rapid deterioration of the fruit. The early harvested fruits of watermelon may

be suitable for the laboratory experiment. As indicated Biokou *et al*. (2015), cucurbit flies attack all stages of growth but prefer small diameter fruits (<10cm) which we couldn't get at the time of our experiment. In terms of pupal production, two varieties of zucchini *caserta* and *magda* gives highest number of pupae. The subsequent adult emergence rate varied among host fruit. Adult emergence rate fluctuated on all vegetable fruits with the highest (100%) on papaya. Comparing *D. vertebratus* emergence rates obtained with data reported in other studies, showed similarity. For instance, Mkiga and Mwatawala, (2015) indicated that highest emergence rate was with pupae collected from pumpkin at 30ºC with low rate at 20ºC. Hence, in all cucurbitaceous hosts, emergence rate increased with the increase of the temperature.

Data obtained indicate that during egg laying periods, adult's longevity is affected by different vegetable host fruit. At both temperature, *D. vertebratus* females live longer than males and for about 20 days on variety *magda* while the shortest longevities time of 17 days (male) and 18 days (female) were observed on watermelon. The adult longevity was significantly affected by vegetable host fruits. Similarly, Patel and Patel, (1998) when they reared *D. ciliatus* on *Coccinia grandis* had noted that adult longevity was 13-27 days.

Of the eight vegetable host fruits, the three variety of zucchini, had high infestation rate at both temperature with the highest rate recorded at 31 ± 1ºC on the variety *magda.* As parents get old, the infestation rate decreased progressively with a lightly increase in older age at 31 ± 1ºC. While at 25 ± 1ºC, vegetable fruits were more infested when parents were at under 18 days old. High infestation rate of *D. vertebratus* in all varieties of zucchini shown its preference to this vegetable fruit with more suitability to the variety *magda*. We conclude that all vegetables fruits including: cucumber, pickle and three varieties of c*aserta,* magda and *fordhook* and papaya are susceptible to be infested by *D. vertebratus* with more preference to the varieties of zucchini. The variety *magda* is more suitable for the mass production of *D. vertebratus*. These findings are in general agreement with the fact that among the tephritid fruit flies infesting the cucurbits, *Dacus* genus, was represented by *D. ciliatus*, *D. bivitattus* and *D. vertebratus* (Gnanvossou *et al.,* 2005; Ekesi and Billah, 2007). The development time and the adult emergence were significantly affected by the parental age under two protein-based diets. However, the tested protein-based diets have no

effect on all observed parameters. Even though the number of collected pupae was higher when adults fed on the mixture of brown sugar and yeast. Parental ages conversely affect the pupae production. As parents get mature, the number of pupae decreased. Similar trends were observed in the total development time. Even though papaya is not a cucurbit's fruits, it has been used as alternative host in the laboratory for the mass rearing of many fruits fly's species such *Bactrocera dorsalis*, *Ceratitis* species.

Along with host susceptibility and suitability of *D. vertebratus* to eight vegetables hosts' fruits, similar experiment was conducted with *Z. cucurbitae*. Three vegetables including, watermelon, zucchini var. *magda* and papaya were used to assess host preference of *Z. cucurbitae.* The longest development time of *Z. cucurbitae* and *Dacus ciliatus* Loew (egg, larvae and pupae) was recorded in pumpkin while the shortest was in cucumber (Vayssières *et al.,* 2008). However, some authors did not used *C. lanatus* as comparative host which is a preferential hosts for *Z. cucurbitae.* This study showed the longest larval development time in *Cucurbita pepo* (6.13 and 6.09 days) with the shortest in *Citrullus lanatus* (5.91 days). However, larval development time was not significantly different among vegetable host fruits and the condition in which parents were found. Hollingsworth *et al.* (1997) reported a range of 3-21 days for larval duration. Furthermore, pupal stage duration ranged from 6.97 days to 9.15 days in *Cucurbita pepo* which are between an averages of 6.50 to 21.80 days on *Lagenaria siceraria* (Koul and Bhagat, 1994). Total developmental time was from 12.83 to 14.75 days at 25 ºC in cucumber which was shorter than 17.20 days (Mkiga and Mwatawala, 2015) and 17.80 days (Huang and Chi, 2012). The difference may be due to the fruit maturity and weight. The highest number of pupae was collected from *Cucurbita pepo* (63.06 to 115.79 pupae) followed by *Citrullus lanatus*. Pupal production was significantly different among vegetable host fruits.

However, the weight and size of pupae collected from *Cucurbita pepo* were significantly lower than those collected from *Citrullus lanatus.* Adult emergence rate was higher on *Citrullus lanatus* with an average of 58.17% and 69.00% with more males' adults than females while more females emerged from *Cucurbita pepo* and *Carica papaya*. Percentage of adults capable of flight from *Cucurbita pepo* was higher than that of the other vegetable fruits in both choice and no choice conditions. All

vegetables hosts tested affected almost in a similar way the developmental time and pupal production of *Z. cucurbitae*. However, even though watermelon remains the preferred hosts, *Cucurbita pepo* and *Carica papaya* are alternative hosts for mass production of the pest in laboratory as they are available throughout the year and affordable.

The application of chemical insecticides lead mainly to resistance, residues in fruits, hasard effects on environment and human health has prompted the development of more suitable strategies of pest control. This implies the reduction of chemical inputs in agriculture in order to provide eco-friendly pest management techniques. Thus, the use of bio-control agents is known as the best alternative to chemical pesticides (Dhaliwal *et al.,* 2012). Entomopathogenic micro-organisms are successful alternative to chemical pesticides in organic agriculture (Glare *et al*., 2012). However, the virulence of the fungus is prior characteristic for entomopathogenic fungi strain selection. They are naturally occurring micro-organisms and are have less harmful effect on the environment. The search for new fungi with entomopathogenic potential in the present work has showed local strains which can be adopted for the control of Cucurbits fruit flies in their local community. In this study, four species of fungi belonging to genera (*Aspergillus*, *Bostryotricum*, *Fusarium*) and an antibiotic, *Streptomycin* were encountered regardless the crops, the isolation methods used and the farming system. This is in line with findings by Omoloye *et al.* (2015) who have recorded a total of seven fungi species with potential as entomopathogenic fungi which include: *A. terreus, A. flavus, A. niger, A. ochraceus, Trichoderma* sp*., Penicillium* sp. and *Rhizopus* sp**.** from different soil samples. Again, findings from others revealed the abundance and occurrence of fungi from selected agricultural fields whereby about 15 species from six genera were isolated from soil and plated on PDA (Gaddy *et al.,* 2012), which corroborate with this study. The most common were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium chrysogenum*, *P. funiculosum*, *Trichoderma viride*, *T. harzianum*, *Fusarium oxysporum*, *F. solani*, *Curvularia clavata*, *Rhizopus stolanifer*. were isolated and characterized from agricultural fields at Salur Mandal (Gaddy *et al.,* 2012). Fungi diversity may be influenced by the farming systems. In this study, fungal species were more abundant in soil samples collected from organic farming than soil treated with chemical pesticides. This suggests that the types of farming in which fungi species are found plays an important role. For instance, organic farming practices have

developed over the years worldwide due to the low inputs of chemicals and their benefits to crop health and the environment (Reganold and Wachter, 2016; van Bruggen *et al.,* 2016). These practices directly affect the abundance and diversity of soil microbial enhancing plant growth and yields, and improved plant resistance to stresses (Kniss *et al.,* 2011). Conversely, conventional farming system relies on higher inputs of synthetic chemicals which affect endophytic fungal.

The present results demonstrate that fungal species' diversity and abundance differed across different cropping systems. Taken as a whole, results indicate that some fungal communities were more abundant among species of crops. This suggests that many inscets could easily be managed with a local entomopathogenic if properly harnessed (Sapna *et al.,* 2012). Our findings showed that the most abundant fungi were *Aspergillus* and *Fusarium* species and could also be readily obtained locally. Species of *Aspergillus* found during our prospection may be produced in the laboratory on artificial media. Results from Yang *et al.* (2015) study have shown the effectiveness of three strains of *Aspergillus*. It has shown that the *A. tamarii* (BC-212) was efficient for the control of melon flies. This corroborated with some recent reports on *Aspergillus* sp. used to reduce pest population. *Aspergillus ochraceus* has been tested and significantly reduced the egg hatching of *Ceratitis capitata* Wiedemann (Castillo *et al.,* 2000). According to works done by Yuan *et al.* (2013), *A. flavus* and *A. tamari* induced the death of melon flies in a period of time with its LC50 against *B. cucurbitae* adults closer to that of *Beauveria bassiana*. However, the lack of larvae and pupae directly from soil throughout the prospection may justify the absence of *Beauveria* and *Metarhizium*. Those fungi are frequently used in the control of fruit fly's species (Vega *et al.,* 2009). It suggests pursuing the investigations and ensure that vegetables fruits were well infested by Tephritid flies through mass trapping.

The most commonly entomopathogenic fungi used are *B. bassiana* and *M. anisopliae.* In this study, only three isolates (MaD, Bb337 and Bb338) caused high death of *Z. cucurbitae* with fungal growth on the cadavers. All isolates have significant larvicidal, pupacidal and adulticidal effect against *Z. cucurbitae.* They induced mortality and sporulation on all host instars development of *Z. cucurbitae* at different concentrations. They have effect even at very low concentrations (10^5 conidia/mL) whereby isolates caused mortality in host instars treated as well as in enclosed host instars. Therefore,

they demonstrate their efficacy against this insect pest under laboratory conditions. These strengthen the fact that fruit flies are vulnerable to entomopathogenic fungi as shown by many authors (Lezama *et al.,* 2000; Castillo *et al.,* 2000).

The present study targeted the third instar larvae, pupae and adults because fruit flies' early instars development is completed within the host fruit. Thus, they are not accessible for direct contact. Most studies used adults in their bioassays which justified the fact that fungal spores are unable to infect directly eggs or larvae usually protected by plant tissues (Brabbs *et al.,* 2015). The effect of fungi isolates is consistent and persisted over the time. Although, all tested fungi were efficacious to the fruit fly species, their virulence varied considerably. For instance, in larvae inoculation methods, isolates Bb337, Bb338 and Bb339 caused up to 20% larval mortality with lowest mortality recorded with isolate MaD. However, the relatively low larval mortality was compensated by a high pupae mortality with different treatments. This result evidently showed the virulence of Bb337, Bb338 and Bb339 which varied depending on fungal species, host instars stages and applied doses as compared to other treatments. Mortality in host instars was not dependant on the concentrations of conidia applied. The present results are in contradictions with conclusions from Mehinto *et al.* (2014) in which mortality was dose-dependent whereby it becomes more significant as the dose increased. Meanwhile in contrast, sporulation rates after treatments were dose dependent. About 60% and over 40% of larval cadavers infected by Bb337 and Bb338 respectively showed fungal activity through sporulation. This confirmed the virulence of those isolates to the larvae of *Z. cucurbitae* and showed that mortality was caused by fungi tested.

Enclosed pupae from inoculated larvae were however highly affected by isolates. Isolate MaD even though has registered the lowest larval mortality, has induced 49.29 to 63.10% pupal mortality. Isolates Bb338 was effective as pupae mortality rate of 35.48 to 49.17% were registered in comparison to isolate Bb337 which showed a rate of 33.95 to 47.49%. This suggests that since enclosed pupae filled to emerge within 15 days after pupation and were fully covered with white mycelium after incubation, the inoculum picked up during larval stage was enough to kill the insect within the mentioned time. Also, pupae mortality is dose-dependent since at the concentration of conidia decrease, the rate of dead pupae decreases. Enclosed pupae with mycosis

followed the same trend which considerably decrease which the decrease in applied dose. A similar persistence of entomopathogenic fungi transmission along the development stages were recorded in pupae with 100% mortality for med-fly and for other insect species (Imoulan and Elmeziane, 2014). Again, highest emergence rate was observed in controls. Isolates significantly reduced subsequent adult emergence. These results demonstrated that once the larval stage of the fruit fly is infected, it is likely to caused mortality in the following host instar development and that reduced considerably the population of the pest at any level of its development. There was a transfer of inoculum from one stage to another and a transmission of infection through cuticle. This explained the fact the infection process lasts with the duration of the host' life cycle increasing the pathogen effect among the population (Beris *et al.,* 2013). It was noticed that emerged adult flies from enclosed pupae died within 3 to 5 days and that dead adults showed fungal growth (up to 18% sporulation rate with Bb338) after incubation. However, most of fruit fly's species acquired sexual maturity. This demonstrated that emerged adults might have not copulate within that time in order to produce eggs for next generation. Isolates Bb337 and Bb338 demonstrated effect on adults of *Z. cucurbitae* when applied directly with sporulation rate of 28.84% and 36% respectively. The results lined with that of Dimbi *et al.,* (2003) recorded adult mortality in *Ceratitis capitata* and *Ceratitis rosa var. fasciventris* (Karsch) treated with several isolates of *B. bassiana* and *M. anisopliae* were 7.00 to 100% and 11.40 to 100% respectively, at four days post inoculation. These results were in conformity with that of Muñoz (2000), wherein 16 strains of *B. bassiana* were assessed on adults of *C. capitata*, mortality was ranged between 20.00 to 98.70%. Also, Quesada-Moraga *et al.* (2006) found 30-100% mortality after 20 days, while testing 10 isolates of *B. bassiana* and five isolates of *M. anisopliae* against adult fruit fly. Sookar *et al.,* (2008) reported the effectiveness of isolates of *M. anisopliae* (07), *B. bassiana* (05) and *Paecilomyces fumosoroseus* (Wise) (02) in adults of *Bactrocera zonata* (Saunders) and *B. cucurbitae* (Coquillett). The susceptibility of adult flies to fungal conidia was also confirmed from different authors (Dimbi *et al.,* 2003; Bahar *et al.,* 2011).

The genotoxicity of two selected isolates of *B. bassiana* Bb337 and Bb338 was assessed by exposing onions bulbs to different concentrations (25%, 50%, 100%, 200% and 300%). Onion (*A. cepa*) roots exposed to distilled water (negative control) and distilled tween 80 water (positive control) for 72 h had both an average number of

roots of 30.60 and showed normal morphology. The number of roots decreased as their concentrations increased. Recommended concentrations (100% of the solution) had an average root reduced by 56.21% for *Bb337* and 47.06% for *Bb338*. However, all the roots were whitish in colour, vertical or oblique in forms, unbroken, straight and aquatic lives. This suggests that the growth of roots might be delayed due to a concentration of conidia suspensions and that it took more times for roots cells to grow up. The fact that none of the tested concentrations caused no morphological abnormalities in the form of hook-shaped roots confirmed that isolates had no toxic effects regardless the concentrations used. This is in concordance with findings of Radic *et al.* (2010) which recorded that toxic effect of a substance may be justified by the presence of tumours and a brown colouration in roots causing morphological abnormalities. The results shown 50% tested concentration had the highest root growth length for Bb3337 (2.18 ± 0.11cm) and Bb338 (2.08 ± 0.12cm) both with the second highest number of roots. It was followed by 25% tested concentration for Bb337 (2.12 ± 0.12) and Bb338 (1.89 ± 0.13) with second highest root growth length and recorded the second highest number of roots. Lastly, at 300% tested concentration had the least root growth (0.44 ± 0.0.16 cm) from Bb337 and (0.37 ± 0.20) in Bb338 also recorded the fewer number of roots (8.60 ± 2.51) from Bb337 and (5.58 ± 1.80) for Bb338. The roots length significantly differed among concentrations and reduced in length as the concentrations increased.

Dacus vertebratus ranked first as the most destructive insect pest among pests causing damaged to watermelon. The high fruit fly infestation might be associated to lack of appropriate control measures. The effectiveness of *B. bassiana* Bb337 and Bb338 was compared to those of a biopesticide ICIPE-69, an isolate of *M. anisopliae* MaD and a chemical pesticide K-Optimal. Spiders and ants' nests were abundant in the control and plots treated with all treatments except the chemical pesticide. Even though, plots were treated twice during rainy season, entomopathogenic based treatments have little effect on their abundance and occurrence. The presence of ants could have also contributed in the reduction of fruit flies' larval stages.

Weekly monitoring of fruits samples showed a progressive reduction in the fruit fly infestation during dry season when treatments were applied once a week while during rainy season, infestation has dropped drastically with frequent application. The

reduction of infestation level can therefore be related to the different treatments of entomopathogenic fungi used. This agrees with Cugala *et al.* (2009) who notified that use of biological control (*M. anisopliae*), protein bait and installed Methyl Eugenol baited traps reduced fruit fly infestation by 93.5 percent in Mozambique. Young fruits of watermelon were highly infested during monitoring. Adult females preferred lay their eggs in young fruits (Ronald and Kessing, 2007). Infestation rate recorded after fruits harvesting and incubation showed variation among treatments and seasons. This may be justified by the fact that maggots feed on the pulp of the fruit which makes is control difficult. Fruit flies injuries represent the greatest contributor to harvest rejections as compared to other causes such as: rotten, cracking, caterpillar infestation. In this study, the highest percent fruits damaged by fruits flies were recorded in the control, 53.08% and 20.56% respectively in dry and rainy seasons. In the treated plots, fruits damaged by fruits flies were important when treatment Bb337 were applied (47.98% and 17.35%). It was followed by Bb338 (35.48% and 11.12%). However, treatments MaD and ICIPE-69 performed better by reducing the level of infestation much lower than *B. bassiana* treatments. In addition, by applying different treatments twice a week, the percent of marketable fruits was increased showing more than 90% undamaged fruits as compared to total harvested weight. Yield of harvested fruits that can be commercialized in terms of size and quality increase when infestation by fruit flies is effective.

CHAPTER SIX

CONCLUSION AND RECOMMANDATIONS

6.1 Conclusion

The present study proved that the management of fruit fly species strongly relies on the mass production in the laboratory which implies the knowledge on its life history parameters. It was concluded that significant variations in the effect of vegetables host fruit, parental ages and temperature was encountered on biological aspects of *D. vertebratus* and *Z. cucurbitae*. Both species preferred watermelon but can complete their life cycle on other vegetables host fruits. *Cucurbita pepo* can be used as alternative host fruit for the mass rearing of both species. Again, adult diet made with the mixture of white sugar and soya-bean which is locally made and cost effective may be used as an alternative to the mixture of brown sugar and yeast.

Cucurbits fruits flies may be controlled using available local biota such as species belonging to the genera, *Aspergillus*, *Botryotricum*, *Fusarium*. Their occurrence and abundance varied among organic and conventional farming practices and among crops grown with soils. It was found that the use of chemical negatively affected their occurrence and diversity. Common entomopathogens used to manage field pests are *B. bassiana* and *Metarhizium.* Although these entomopathogenic fungi were not detected during the prospection made in this study, any presumption may not be precluded regarding its possible incorporation as biocontrol agent against fruit flies. Till, literature overflow on its effectiveness against fruit flies. This suggest pursuing the investigations and ensure that vegetables fruits were well infested by Tephritid flies through mass trapping. In this study, three isolates of *B. bassiana* and *Metarhizium* (MaD, Bb337 and Bb338) were found to have larvicidal, pupacidal and adulticidal effect against *Z. cucurbitae* and *D. vertebratus*. They induced mortality and sporulation on all host instars development of *Z. cucurbitae* and *D. vertebratus* at different concentrations. Therefore, they demonstrated their efficacy against this insect pest under laboratory conditions. Also, we found out that once the larval stage of the fruit fly is infected, it is likely to cause mortality in the following host instar

development reducing considerably the population of the pest at any level of its development. Entomopathogenic fungi isolates have proved to have no toxic effects regardless the concentrations used.

Dacus vertebratus causes important damage in watermelon in preference in young fruits. The use of chemical pesticide however negatively affects the abundance and occurrence of some beneficial insects such as ants and spiders. Fruit flies damage have as consequences the rejection of fruits. Application of bio-pesticide based on *Metarhizium* and *Beauveria* gives high yield of fruit in size and quality reducing infestation of fruit flies in watermelon. Although, there are more likely easy to prepare and are efficient to suppress fruit fly, it requires regular applications owing to more labour cost. However, spraying chemical insecticide is worthless in fruit fly management options and caused more adverse to beneficial insects and environment. After all, the use of ecological approaches using entomopathogenic fungi is found to be effective and could be ecologically sound.

6.2 Recommendations

The native biota could be utilised for the suppression of fruit flies which suggest that prospection should be conducted through larvae and pupae samples collection from soil for fungi isolation. Nevertheless, further studies would be necessary to assess the effectiveness of these locally sourced potential biocontrol agents against host instar development of fruit flies under the screenhouse and field conditions. The use of conventional pesticide should be restricted to environmentally friendly alternative in order to prevent dexterous effect on the native entomopathogenic species. Suggesting that fungi species such as *Aspergillus niger*, *A. flavius*, *Fusairum* sp. should be ecologically conserved. Ecological approach to management of fruit fly's species is feasible using *B. bassiana* species. Although the isolates from Senegal (MaD) and from Kenya (ICIPE-69) reduced the level of infestation much lower than *B. bassiana* treatments; other local isolates of *B. bassiana* should be explored. The formulation of the isolates of *B. bassiana* in biopesticide should be considered in other to provide an alternative to the use of chemical.

REFERENCES

Adandonon, A., Vayssières, J. Sinzogan, A. and Van Mele, P. 2009. Density of pheromone sources of the weaver ant *Oecophylla longinoda* affects oviposition behaviour and damage by mango fruit flies (Diptera: Tephritidae). *International Journal of Pest Management* 55.4:285-292.

Afari-Sefa, V., Rajendran, S., Kessy, R. F., Karanja, D. K., Musebe, R., Samali, S. and Makaranga, M. 2016. Impact of nutritional perceptions of traditional African vegetables on farm household production decisions: A case study of smallholders in Tanzania. *Experimental Agriculture* 52:300-313.

AGRA (Alliance for a Green Revolution in Africa). 2016. Africa agriculture status report 2016. Progress towards agricultural transformation in Africa. Retrieved Oct. 3, 2019, from https://reliefweb.int/report/world/africa-agriculture-status-report-2016-progress-towards-agricultural-transformation

Al-Masri, M. 2015. Effectiveness of *Beauveria bassiana* Native isolates in the Biological Control of the Mediterranean Fruit Fly (Ceratitis capitata). *Advances in Entomology* 3:44-55.

Altendorf, S. 2019. Major tropical fruits market review 2018. Rome, FAO.18p. Retrieved Oct. 3, 2019, from http://www.fao.org/3/ca5692en/CA5692EN.pdf.

Amao, I. 2018. Health Benefits of Fruits and Vegetables: Review from Sub-Saharan Africa. Vegetables: Importance of Quality Vegetables to Human Health, 33.

Andolfo, G., Di-Donato, A., Darrudi, R., Errico, A., Aiese Cigliano, R. and Ercolano, M. R. 2017. Draft of zucchini (*Cucurbita pepo* L.) proteome: A resource for genetic and genomic studies. *Frontiers in genetics* 8, 181.

Aniekwe, L. and Nwokwu, G. 2013. Effects of Organic Manure Sources on the Growth and Yield of Watermelon in Abakaliki, Southeastern Nigeria. International Journal of Science and Research 2319-7064.

Allwood, A. J., Vueti, E. T., Leblanc, L. and Bull, R. 2002. Eradication of introduced *Bactrocera* species (Diptera: Tephritidae) in Nauru using male annihilation and protein bait application techniques, pp. 19-25. In Turning the tide: The eradication of invasive species: *Proceedings of the International Conference on eradication of island invasives* (VEITCH C. R., CLOUT M. N., Eds), Auckland, New Zealand.

Aluja, M. and Mangan, R. L. 2008. Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological and regulatory considerations. *Annual Review of Entomology* 53:473-502.

Amur, A., Memon, N., Shah, M. A., Ansari, A., Solangi, D. A., and Mal, B. 2017. Biology and morphometric of different life stages of the oriental fruit fly *(Bactrocera Dorsalis* Hendel) (Diptera: Tephritidae) on three varieties of mango of sindh, Pakistan. *JAPS: Journal of Animal and Plant Sciences* 27.5.

APHIS-USDA, 2009. Federal import quarantine order for host materials of *Bactrocera invadens* (Diptera: Tephritidae), invasive fruit fly species, 5 pp.

Arora, R. and Shera, P. S. 2014. Genetic improvement of biocontrol agents for sustainable pest management. In *Basic and applied aspects of biopesticides* (pp. 255-285). Springer, New Delhi.

Badii, K. B., Billah, M. K., Afred-Nuamah, K., Obeng-Ofori, D. and Nyarko, G. 2015. Review of the pest status, economic impact and management of fruit-infesting flies (Diptera: Tephritidae) in Africa. *African Journal of Agricultural Research* 10.12:1488-1498.

Bahar, M. H., David, B., Peter, C. G. and Robert, M. 2011. Efficacy of a *Cladosporium* sp. fungus against *Helicoverpa armigera* (Lepidoptera: Noctuidae), other insect pests and beneficial insects of cotton. *Biocontrol Science Technology* 21:1387-1397.

Bárbara, R., Mora, L., Oliveira, M. D., Euler, A. C., Larav, L., and Lezcano, P. 2011. "Chemical Composition and Nutritive Value of Torula Yeast (Candida utilis), Grown on Distiller's Vinasse, for Poultry Feeding." *Cuban Journal of Agricultural Science* 45.2:261.

Barclay, H. J., McInnis, D. and Hendrichs, J. 2014. Modeling the area-wide integration of male annihilation and the simultaneous release of methyl eugenol-exposed *Bactrocera* spp. sterile males. *Annals of the Entomological Society of America* 107.1:97-112.

Bateman, R. P., Carey, M., Batt, D., Prior, C., Abraham, Y., Moore, D., Jenkins, N. and Fenlon, J. 1996. Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskål). *Biocontrol Science and Technology* 6:549-560.

Benelli, G. 2015. Aggression in Tephritidae flies: where, when, why? Future directions for research in integrated pest management. *Insects* 6.1:38-53.

Ben-Yosef, M., Pasternak, Z., Jurkevitch, E. and Yuval, B. 2015. Symbiotic bacteria enable olive fly larvae to overcome host defences. *Royal Society Open Science* 2:150-170.

Beris, E. I., Papachristos, D. P., Fytrou, A., Antonatos, S. A. and Kontodimas, D. C. 2013. Pathogenicity of three entomopathogenic fungi on pupae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Pest Science* 86:275-284.

Bianchi, F. A. and Krauss, N. H. 1937. Fruit fly investigations in East Africa. *Hawaiian Planters Record* 41:299-306.

Biokou, M. M., Adjakpa, J., Bokonon-Ganta, A., and Karlson, M. 2015. Evaluation de la vulnerabilite de quatre varietes de pasteque *Citrullus lanatus* (Thunb.) Matsum.

and Nakai, à la mouche *Dacus vertebratus* (Diptera: Tephritidae). Ecole Polytechnique de Abomey-Calavi/Universite d'Abomey Calavi (EPAC/UAC).

Boisclair, J. and Estevez, B. 2006. Lutter contre les insectes nuisibles en agriculture biologique: intervenir en harmonie face à la complexité. *Phytoprotection* 87.2:83-90.

Bokonon-Ganta, A. H., Noudofinin, M., Hanna, R. and Gnanvossou, D. 2010. Les mouches nuisibles aux fruits et légumes fruits: importance économique et approches de lutte. Fiche technique d'information et d'éducation. Dépôt légal n°4935 du 10 /12/2010 4ème trimestre Bibliothèque Nationale du Bénin, 15p.

Boucias, D. G., Mazet, I., Pendland, J. and Hung, S. Y. 1995. Comparative analysis of the in vivo and in vitro metabolites produced by the entomopathogen *Beauveria bassiana*. *Canadian journal of botany* 73.1:1092-1099.

Brabbs, T., Collins, D., Hérard, F., Maspero, M. and Eyre, D. 2015. Prospects for the use of biological control agents against *Anoplophora* in Europe. *Pest Management Science* 71:7-14.

Braga, G. U. L., Flint, S. D., Miller, C. D., Anderson, A. J., Roberts, D. W. 2001. Variability in response to UV-B among species and strains of *Metarhizium* isolated from sites at latitudes from 61°N to 54°S. *Journal of Invertebrate Pathology* 78.2: 98–108.

Bugeme DM, Maniania NK, Knapp M, Boga HI. 2008. Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. *Experimental and Applied Acarology* 46.1-4: 275–285.

CABI, (2017). Invasive Species Compendium. Retrieved online October 2019 from http://www.cabi.org/isc/

Capuzzo, C., Firrao, G., Mazzon, L., Squartini, A. and Girolami, V. 2005.'Candidatus Erwinia dacicola', a co-evolved symbiotic bacterium of the olive fly *Bactrocera oleae* (Gmelin). *International Journal of Systematic Evolutionary Microbiology* 55:1641-1647.

Carey, J. R., Papadopoulos, N. and Plant, R., 2017. The 30-year debate on a multi-billion-dollar threat: tephritid fruit fly establishment in California. *American Entomology* 63:100-113.

Carrillo, D., Duncan, R. E., Ploetz, J. N., Campbell, A. F., Ploetz, R. C. and Peña, J. E. 2014. Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. *Plant Pathology* 63.1:54-62.

Caroll, E. L., White, I. M., Freidberg, A., Norrbom, A. L., Dallwitz, M. J. and Thompson, F. C. 2002. Pest fruit flies of the world. Version: 2nd April 2019. delta-intkey.com.

Castillo, M. A., Moya, P., Hernandez, E. and Primo-Yufera, E. 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biological Control* 19:274-282.

Charnley, A. K. 1989. Mechanisms of fungal pathogenesis in insects. *In:* The Biotechnology of fungi for improving plant growth. Eds. Whipps, J. M., Lumsden, R. D.), Cambridge University, London. pp.85-125.

Charnley, A. K. and Collins, S. A. 2007. Entomopathogenic Fungi and Their Role in Pest Control. *Environmental and microbial relationships* 4:159.

Chandini, K. and Rajeshwari, N. 2017. Isolation and identification of soil fungi in Mattavara forest, Chikamagalur, Karnataka. J*ournal of Pharmacognosy and Phytochemistry* 6.5: 721-726.

Chang, C. L. 2009. Fruit fly liquid larval diet technology transfer and update. Journal of Applied Entomology 133:164–173.

Clarkson, J. M. and Charnley, A. K. 1996. New insights into the mechanisms of fungal pathogenesis in insects. *Trends in microbiology* 4.5:197-203.

Coquillett, D. W. 1899. A new trypetid from Hawaii. *Entomological News* 10:129-130.

Cowley, J. M., Baker, R. T. and Harte, D. S. 1992. Definition and determination of host status for multivoltine fruit fly (Diptera: Tephritidae) species. J*ournal of Economic Entomology* 85.2:312-317.

Cugala, D., Jose, L., Cocorrea, H. and Ekesi, S. 2012. Assessing the impact of integrated pest management programme for suppressing the invasive fruit fly (Bactrocera invadens) on mango in Mozambique. In Third RUFORUM Biennial Meeting, Entebbe (pp. 301-306).

Deguine, J. P., Atiama-Nurbel, T., Aubertot, J. N., Augusseau, X., Atiama, M., Jacquot, M. and Reynaud, B. 2015. Agroecological management of cucurbit-infesting fruit fly: a review. *Agronomy for sustainable development* 35.3:937-965.

De la rosa, W., Alatorre, R., Barrera, J. F. and Toriello, C. 2000. Effect of *Beauveria bassiana, Metarhizium anisopliae* (Deuteromycetes) upon the coffee berry borer (Coleoptera: Scolytidae) under field conditions. *Journal of Economic entomology* 93.5:1409-1414.

de Meyer, M., Delatte, H., Mwatawala, M., Quilici, S., Vayssières, J-F and Virgilio, M. 2015. A review of the current knowledge on *Zeugodacus cucurbitae* (Coquillett) (Diptera, Tephritidae) in Africa, with a list of species included in *Zeugodacus*. *ZooKeys* 540:539–557.

_____, Quilici, S., Franck, A., Chadhouliati, A. C., Issimaila, M. A., Youssoufa, M.A., Barbet, A., Attié, M. and White, I. M. 2012. Frugivorous fruit flies (Diptera, Tephritidae, Dacini) of the Comoro Archipelago. *African Invertebrates* 53: 69-77.

de Souza, E. O., Anato, F. M., Bokonon-ganta, A. H. et Bokonon-ganta, E. B. 2016. Contraintes à l‟adoption de la méthode de l‟hygiène sanitaire des vergers pour la lutte contre les mouches nuisibles aux fruits (Diptera, Tephritidae) par les producteurs de mangues et d‟agrumes au Benin. *Agronomie Africaine* 28.1:60-69.

Devi, K. U., Sridevi, V., Mohan, Ch-M., Padmavathi, J. 2005. Effect of high temperature and water stress on in vitro germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. *Journal of Invertebrate Pathology* 88.3: 181-189.

Dhaliwal, G. S., Koul, O., Khokhar, S. and Singh, R. 2012. Biopesticides: Springboard to environment and food security. In Koul, O., Dhaliwal, G. S., Kokhar, S., Singh, R. (eds.), Biopesticides in Environment and Food Security: Issues and Strategies, Scientific Publishers (India) Jodhpur, 1-11.

Dimbi, S., Maniania, N. K. and Ekesi, S. 2009. Effect of *Metarhizium anisopliae* inoculation on the mating behavior of three species of African Tephritid fruit flies, *Ceratitis capitata*, *Ceratitis cosyra* and *Ceratitis fasciventris*. *Biological Control* 50.2:111-116.

_____, Maniania, N. K., Lux, S. A., Ekesi, S., and Mueke, J. K. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). *Mycopathologia* 156.4:375-382.

Dohino, T., Hallman, G., Grout, T. G., Clarke, A. R., Hernandez, E., Follett, P. A., Cugala, D. R., Tu, D. M., Murdita, W., Myers, S. W. and Pereira, R. 2016. Phytosanitary Treatments Against *Bactrocera Dorsalis* (Diptera : Tephritidae): Current Situation And Future Prospects. *Journal of Economic Entomology* 100.1:1-13.

Doorenweerd, C., Leblanc, L., Norrbom, A. L., San Jose, M. and Rubinoff, D. A. 2018. Global checklist of the 932 fruit fly species in the tribe Dacini (Diptera, Tephritidae). *ZooKeys* 730:17-54.

_____, C., Leblanc, L., Hsu, Y-F., Huang, C-L., Lin, Y-C., San Jose, M. and Rubinoff, D. 2019. Taiwan's Dacini Fruit Flies: Rare Endemics and Abundant Pests, along Altitudinal Gradients. *Pacific Science* 73.1:35-59.

Drew, R. A. I. 1973. Revised descriptions of species of Dacini (Diptera: Tephritidae) from the South Pacific area. I. Genus Callantra and the Dacus group of subgenera of genus Dacus. Bulletin of the Queensland Department of Primary Industries, Division. *Plant Industries* 652:1-39.

_____. 1989, Taxonomy and distribution of tropical and subtropical Dacinae (Diptera: Tephritidae). In: Robinson S.A., Hooper G. (eds), World Crops Pests 3(A). Amsterdam, Elsevier: 13-66.

_____. and Romig, M. C. 2013. Tropical fruit flies (Tephritidae: Dacinae) of South-East Asia: Indomalaya to North-West Australasia. CAB International Wallingford, Oxfordshire, GB, 653 pp.

Duyck, P. F. 2005. Compétition interspécifique et capacité invasive, le cas des Tephritidae de l'ile de la Réunion. Thèse de diplôme de Doctorat. Université de la Réunion. Faculté des Sciences et Technologie, 103 p.

_____, David, P. and Quilici, S. 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). *Ecological Entomology* 29.5:511-520.

Edwards, A. J., Vinyard, B. T., Wiley, E. R., Brown, E. D., Collins, J. K. and Perkins-Veazie, P. 2003. Consumption of watermelon juice increases plasma concentrations of lycopene and β- carotene in humans. *Journal of Nutrition* 133:1043-50.

Ekesi S, Mohamed S and Hanna R, 2010. Rid fruits and vegetable in Africa of notorious fruit flies. *Technical Innovation Brief* 4:2.

_____, Maniania, N. K., Ampong-Nyarko, K. 1999. Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti*. *Biocontrol Science and Technology* 9.2: 177–185

_____, Nderitu P. W. and Chang, C. L. 2007. Adaptation to and Small-Scale Rearing of Invasive Fruit Fly *Bactrocera invadens* (Diptera: Tephritidae) on Artificial Diet. *Annals of the Entomological Society of America* 100:562-567.

_____, Nderitu, P W. and Rwomushana, I. 2006. Field infestation, life history and demographic parameters of the fruit fly Bactrocera invadens (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research* 96.4:379-386.

_____, and Billah, M. 2006. Tephritid Fruit Flies in Africa-Fact Sheets of Some Economically Important Species in A Field Guide to the Management of Economically Important Tephritid Fruit Flies in Africa 13 p.

_____, and Billah, M. K. 2007. A field guide to the management of economically important tephritid fruit flies flies in Africa. 2nd Edition. ICIPE Science Press, Nairobi, Kenya.

_____, and Mohamed, S. A. 2011. Mass rearing and quality control parameters for tephritid fruit flies of economic importance in Africa. In *Wide spectra of quality control*. IntechOpen.

_____, De Meyer, M., Mohamed, S. A., Virgilio, M. and Borgemeister, C. 2016. Taxonomy, ecology, and management of native and exotic fruit fly species in Africa. *Annual Review of Entomology* 102:981-991.

_____, Maniania N. K. and Mohamed S. A. 2011. Efficacy of soil application of

Metarhizium anisopliae and the use of GF-120 spinosad bait spray for suppression of *Bactrocera invadens* (Diptera: Tephritidae) in mango orchards. *Biocontrol Science and Technology* 21:299-316.

Elbashir, M. I., Paul, B. Shankarganesh, K. Gautami, R. and Sharma, P. 2014. Pathogenicity of indian isolates of entomopathogenic fungus against important insect pests and natural enemies. *Indian Journal of Entomology* 76:37-43.

Ellis, B. W., Bradley, F. M. and Atthowe, H. (Eds.). 1996. *The organic gardener's handbook of natural insect and disease control: A complete problem-solving guide to keeping your garden and yard healthy without chemicals*. Rodale.

Enujeke, E. C. 2015. An assessment of some growth and yield indices of six varieties of watermelon (*Citrulus lanatus* thunb.) in Asaba area of Delta state, Nigeria. *Tropical Agricultural Research and Extension* 16.4.

EPPO, G. 2018. EPPO Global database. European and Mediterranean Plant Protection Organization (EPPO). Retrieved on Mar., 24, 2019

Erhirhie, E. O. and Ekene, N. E. 2013. Medicinal Values on *Citrullus lanatus* (Watermelon). *International Journal of Pharmacy and Biomedical Sciences* 4.4:1305-1312.

FAO, 2018. FAOSTAT Database. Food and Agriculture Organization of the United Nations. Retrieved Aug. 13, 2019, from http://www.fao.org/faostat/en.

____, 2017. Crop statistics are recorded for 173 products. Retrieved Jun. 13, 2019, from http://www.fao.org/faostat/en/#data/QC.

FAOSTAT (Food and Agriculture Organization of the United Nations). 2017. Watermelon statistics. Retrieved Jun. 13, 2019, from http:/ /www.fao.org/faostat/en/#data/QC.

Fargues, J. and Luz, C. 2000. Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauveria bassiana* for *Rhodnius prolixus*. *Journal of Invertebre Pathology* 75.3:202-211.

Fernandes, É. K. K., Rangel, D. E. N., Moraes, Á. M. L., Bittencourt, V. R. E. P., Roberts, D. W. 2007. Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. *Journal of Invertebrate Pathology* 96.3:237–243.

_____, Rangel, D. E. N., Braga, G. U. L., Roberts, D. W. 2015. Tolerance of entomopathogenic fungi. *Current Genetics* 61.3:427-440.

Ferron, P., J. Fargues, and G. Riba. 1991. Fungi as Microbial Insecticides against Pests. Pp. 665–706. *In: Handbook of Applied Mycology*. Eds. D. K. Arora, L. Ajello and K. G. Mukerjii Vol. 2, Marcel Dekker, New York.

Filotas, M. J. and Hajek, A. E. 2004. Influence of temperature and moisture on infection of forest tent caterpillars (Lepidoptera: Lasiocampidae) exposed to

resting spores of the entomopathogenic fungus *Furia gastropachae* (Zygomycetes: Entomophthorales). *Environmental entomology* 33.5:1127-1136.

Flores, S., Campos, S., Villaseñor, A., Valle, A., Enkerlin, W., Toledo, J., Liedo, P. and Montoya, P. 2013. Sterile males of *Ceratitis capitata* (Diptera: Tephritidae) as disseminators of *Beauveria bassiana* conidia for IPM strategies. *Biocontrol Science and Technology* 23:1186-1198.

Freidberg, A., Kovac, D., Shiao, S. 2017. A revision of Ichneumonopsis Hardy, 1973 (Diptera: Tephritidae: Dacinae: Gastrozonini), Oriental bamboo-shoot fruit flies. *European Journal of Taxonomy* 317:1-23.

Furlong, M. J. and Pell, J. K. 2005. Interactions between entomopathogenic fungi and arthropod natural enemies. *Insect-fungal associations: ecology and evolution. Oxford University Press, New York*, 51-73.

Fuxa, J. R. and Richter, A. R. 2004. Effects of soil moisture and composition and fungal isolate on prevalence of *Beauveria bassiana* in laboratory colonies of the red imported fire ant (Hymenoptera: Formicidae). *Environmental entomology* 33.4:975-981.

Gaddeyya, G., Niharika, P. S., Bharathi, P. and Kumar, P. R. 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Advances in Applied Science Research* 3.4:2020-2026.

Gaddy, J. A., Arivett, B. A., McConnell, M. J., López-Rojas, R., Pachón, J. and Actis, L. A. 2012. Role of acinetobactin-mediated iron acquisition functions in the interaction of *Acinetobacter baumannii* strain ATCC 19606T with human lung epithelial cells, *Galleria mellonella* caterpillars, and mice. *Infection and immunity* 80.3:1015-1024.

Gallo, J. 2007. Integrated Plant Control: System and management. *Encyclopedia of Pest Management* 2:279-287.

García-Martínez, M. A., Presa-Parra, E., Valenzuela-González, J. E. and Lasa, R. 2018. The Fruit Fly Lure CeraTrap: An Effective Tool for the Study of the Arboreal Ant Fauna (Hymenoptera: Formicidae). *Journal of Insect Science* 18.4:16.

Garcia, F. R. and Ricalde, M. P. 2013. Augmentative biological control using parasitoids for fruit fly management in Brazil. *Insects* 4.1:55-70.

Gilbert, E. 2004. Dhows and the Colonial Economy of Zanzibar, 1860–1970. Ohio University Press, Athens, Ohio, 167 pp.

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J. and Stewart, A. 2012. Have biopesticides come of age?. *Trends in biotechnology* 30.5:250-258.

Gnanvossou, D., Hanna, R. Azandémè, G., Georgen, G. Tindo, M. and Agbaka, A. 2005. Inventaire et importance des dégâts des mouches des fruits sur quelques espèces de cucurbitacées au Benin. Actes de l'atelier Scientifiques national édité

par A. Adjanohoun et K. Igue. Abomey-Calavi 19-22 Décembre 2006, Tome 1. ISBN 978-99919-6850-6, ISSN 1659-6161. Dépôt légal n 3838 du 13/08/2008 3e trimestre, Bibliothèque Nationale du Benin. 140-145 pp.

Grant, A. 2018. Sugar Baby Cultivation, Tips For Growing A Sugar Baby Watermelon Read more at Gardening. Retrieved Aug. 13, 2019, from https://www.gardeningknowhow.com/edible/fruits/watermelon/sugar-baby-cultivation.htm.

Grove, T. and Beer, M. S. D. 2014. Monitoring fruit flies in Litchi orchards in South Africa and determining the presence of alien invasive *Bactrocera* species. *Acta Horticulturae* 1029:425-432

Hadi, M. S., Himawan, T. and Aini, L. Q. 2017. The application of *Beauveria bassiana* and Lufenuron could reduce the reproduction of fruit fly (*Bactrocera carambolae*) (Drew & Hancock) (Diptera: Tephritidae). *Journal of Tropical Plant Protection* 1:22-29.

Hajek, A. E. and St. Leger, R. J. 1994. Interactions between fungal pathogens and insects hosts. *Annual Review of Entomology* 39:293-322.

Hallouti, A., Zahidi, A., Bouharroud, R., El Mousadik, A., Aoumar, A. A. B. and Boubaker, H. 2017. Diversity of entomopathogenic fungi in Argane forest soil and their potential to manage Mediterranean fruit fly (*Ceratitis capitata*). *Journal of Pharmacy and Pharmacology* 5:746-754.

Hasyim, A. Maryati, S. and Kogel, W. J. 2018. Population fluctuation of adult males of the fruit fly, *Bactrocera tau* walker (Diptera: Tephritidae) in passion fruit orchards in relation to abiotic factors and sanitation I*ndonesia Journal of Agricultural science* 9:29-33.

Hedström, P. 1994. Contagious collectivities: On the spatial diffusion of Swedish trade unions, 1890-1940. *American Journal of Sociology* 99.5:1157-1179.

Heve, W. K., El-Borai, F. E., Carrillo, D. and Duncan, L. W. 2017. Increasing entomopathogenic nematode biodiversity reduces efficacy against the Caribbean fruit fly *Anastrepha suspensa:* interaction with the parasitoid *Diachasmimorpha longicaudata*. *Journal of Pesticide Science* 91.2:799-813.

Hintènou, V. M. 2011. Étude comparative du développement de *Bactrocera dorsalis* Drew, Tsuruta et White (Diptera: Tephritidae) sur une plante hôte préférentielle (*Irvingia gabonensis*) et une plante hôte secondaire (*Musa* sp.). Mémoire pour l'obtention du Diplôme de Licence Professionnelle – Université Catholique de l'Afrique de l'Ouest (UCAO). 51 p.

Hollingsworth, R. G., Vagalo, M., Tsatsia, F., Allwood, A. J. and Drew, R. A. I. 1997. Biology of melon fly, with special reference to Solomon Islands. In *ACIAR PROCEEDINGS* (pp. 140-144). *Australian Centre for International Agricultural Research* 76:140-44.

HORTGRO, 2016. Fruit fly Africa: Life cycle of medfly and natal fruit fly. Retrieved May, 2018 from *www.fruitfly.co.za/life-cycle-and-identification/life-cycle/*

Howélé, O., Abdoulaye, T., Vazoumana, K. and Kati-coulibaly, S. 2018. The use of cucurbitaceae fruits and seeds: research synthesis. *International Journal of Agronomy and Agricultural Research* 12.5:47-59.

Huang, Y. B. and Chi, H. 2012. Age-stage, two-sex life tables of *Bactrocera Cucurbita* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. *Insect Science* 19.2:263-273.

ICIPE, 2007. Development and implementation of a sustainable IPM program for major mango pests and opportunity for improving market information and processing in Sub-Saharan Africa. ICIPE Science Press.

IFAD, International Fund for Agricultural Development. 2019. Benin and IFAD to boost food and nutrition security and create jobs for rural people. Retrieved Oct. 1, 2019, from https://www.ifad.org/en/web/operations/country/id/benin.

Ignjatovic, M. 2005. About Watermelons. Watermelon-Serbia.com. Retrieved Feb. 17, 2018 from *http://www.watermelon-serbia.com/english/strainice/olubenicama.htm.*

Imoulan, A. and Elmeziane, A. 2014. Pathogenicity of *Beauveria bassiana* isolated from Moroccan Argan forests soil against larvae of *Ceratitis capitata* (Diptera: Tephritidae) in laboratory conditions. *World Journal of Microbiology and Biotechnology* 30:959-965.

_____, Ibnsouda, S. K. and El-Meziane, A. 2016. Molecular characterization and the effectiveness of native entomopathogenic *Beauveria bassiana* strains against adults of Mediterranean fruit fly (*Ceratitis capitata*). *Journal of Biosciences and Biotechnological Discovery* 1:6-16.

Inglis, G. D., Goettel, M., Butt, T. and Strasser, H. 2001. Use of hyphomycetous fungi for managing insect pests. In: Butt, T. M; Jackson, C.W. and Magan, N. (Eds*.)* Fungi as biocontrol agents: progress, problems and potentials. CABI Publishing, Wallingford, United Kingdom, 23-70.

_____, Duke, G. M., Kawchuk, L. M., Goettel, M. S. 1999. Influence of oscillating temperatures on the competitive infection and colonization of the migratory grasshopper by *Beauveria bassiana* and *Metarhizium flavoviride*. *Biological Control* 14.2:111-120.

Inskeep, J. R., Spafford, H. and Shelly, T. E. 2018. Trapping Male Melon Flies, Zeugodacus cucurbitae (Coquillett) (Diptera: Tephritidae), using mixtures of Zingerone and Cue-Lure in the Field. *Proceedings of the Hawaiian Entomological Society* 50:67-75.

Jallow, M. F. A., Awadh, D. G., Albaho, M. S., Devi, V. Y. and Thomas, B. M. 2017. Pesticide risk behaviour and factors influencing pesticide use among farmers in Kuwait. *Science of the Total Environment* 574:490-498.

Jain, S., Jerald, E. E. and Edwin, S. 2013. Isolation, fractionation and evaluation of the anti-inflammatory properties of *Citrullus lanatus*Thunb. *Asian Journal of Biomedical and Pharmaceutical Science* 3:66-72.

Jang, E. B., McQuate, G. T., McInnis, D. O., Harris, E. J., Vargas, R. I., Bautista, R. C., and Mau, R. F. 2008. Targeted trapping, bait-spray, sanitation, sterile-male, and parasitoid releases in an areawide integrated melon fly (Diptera: Tephritidae) control program in Hawaii. *American Entomologist* 54.4:240-250.

Jaronski, S. T. 2010. Ecological factors in the inundative use of fungal entomopathogens. *BioControl* 55.1:159–185.

Jarret, B., R. Bill, Tom, W. and Garry. A. 1996. Cucurbits Germplasm Report. Watermelon National Germplasm System. pp 29-66 Agricultural Service, U.S.D.A

Kadio, E. A. A, Aboua, L. R. N., Seri-Kouassi, P. B., Koua, K. H., Hala, N. and Vayssières, J. F. 2011. Inventory of parasitoids for a biological control of fruits flies (Diptera: Tephritidae) in Côte d'Ivoire. *Journal of Research Biology* 7:467–476.

Kambura, C., Tanga, C. M., Kilalo, D., Muthomi, J., Salifu, D., Rwomushana, I. and Ekesi, S. 2018. Composition, Host Range and Host Suitability of Vegetable-Infesting Tephritids on Cucurbits Cultivated in Kenya. *African Entomology* 26.2:379-397.

Kassab, S. E., Al-Shafei, A. I., Salem, A. H. and Otoom, S. 2015. Relationships between the quality of blended learning experience, self-regulated learning, and academic achievement of medical students: a path analysis. *Advances in medical education and practice* 6:27.

Khanna, N. and Solanki, P. 2014. Role of agriculture in the global economy. Second International Conference on Agricultural and Horticultural Sciences. Radisson Blu Plaza Hotel, India, February 03-06, 2014. *Agrotechnology* 2:4.

Klasen, S., Meyer, K. M. and Dislich, C. 2016. Economic and ecological trade-offs of agricultural specialization at different spatial scales. *Ecological Economics* 122:111-120.

Kniss, A. R., Vassios, J. D., Nissen, S. J. and Ritz, C. 2011. Nonlinear regression analysis of herbicide absorption studies. *Weed Science* 59:601–610.

Kostantonopolou, M. A. and Mazomenos, B. E. 2005. Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata. BioControl* 50:293-305.

Koul, V. K. and Bhagat, K. C. 1994. Biology of melon fly, *Bactrocera* (Dacus) *Cucurbita*Coquillet (Diptera: Tephritidae) on bottle gourd. *Pest Management and Economic Zoology* 2(2):123–125.

Kounatidis, I., Crotti, E., Sapountzis, P., Sacchi, L., Rizzi, A. and Chouaia, B. 2009 *Acetobacter tropicalis* is a major symbiont of the olive fruit fly (*Bactrocera oleae*). *Applied and Environmental Microbiology* 75:3281-3288.

Krosch, M. N., Schutze, M. K., Armstrong, K. F., Graham, G. C., Yeates, D. K. and Clarke, A. R. 2012. A molecular phylogeny for the Tribe Dacini (Diptera: Tephritidae): Systematic and biogeographic implications. *Molecular Phylogenetics and Evolution* 64.3:513-523.

Lacey, L. A. and Shapiro-Ilan, D. I. 2008. Microbial control of insect pests in temperate orchard systems: potential for incorporation into IPM. *Annual Review of Entomology* 53:121-144.

Laghetti, G. and Hammer, K. 2007. The Corsican citron melon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai subsp. *lanatus* var. citroides (Bailey) Mansf. ex Greb.) a traditional and neglected crop. *Genetic Resources and Crop Evolution* 54.4:913-916.

Leger, R. J. S. and Wang, C. 2010. Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. *Applied Microbiology and Biotechnology* 85.4:901-907.

Lezema, G. R., Trujillo-De, L. A., Molina, A. L., Rebolledo-Dominguez, J., Pescådor, O., LOpez-Edwards, A. R. M. and Aluja, M. 2000. Virulence of *Metarhizium anisopliae* Lezema, G. R., Trujillo-De, L. A., Molina, A. L., Rebolledo-Dominguez, J., Pescådor, O., Lopez-Edwards, A. R. M. and Aluja, M. 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): laboratory and field trials. *Journal of economic entomology* 93:1080-1084.

Lyon, E. E. and Miller, S. E. 2000. Invasive species in Eastern Africa. Proceedings of a workshop held at ICIPE, 5-6 July 1999. ICIPE Science Press, Nairobi P. 108.

Lord, J. C. 2011. Influence of substrate and relative humidity on the efficacy of three entomopathogenic fungi for the hide beetle, *Dermestes maculatus* (Coleoptera, Dermestidae). *Biocontrol Science and Technology* 21.4: 4756-483.

Lovett, B., St. Leger, R. J. 2018. Genetically engineering better fungal biopesticides. *Pest Management Science* 74.4:781-789.

Mahat, K. and Drew, R. A. 2012. Evaluation of protein bait laced with various insecticides on the Queensland fruit fly (Diptera: Tephritidae): attraction, feeding, mortality and bait persistence. In *XII International Citrus Congress-International Society of Citriculture* (pp. 18-23).

Maina, U. M., Galadima, I. B. Gamba, F. M. and Zakaria, D. 2018. A review on the use of entomopathogenic fungus in the management of insect pest of field crops. *Journal of Entomology and Zoology Studies* 6:27-32.

Manrakhan, A. 2016. Fruit fly.Citrus Research International. Nelspruit, South Africa.

McCoy, C.W., Samson, R. A. and Boucias, D. G. 1988. *Entomogenous fungi. In*: CRC Handbook of Natural Pesticides: Microbial Insecticides. Part A. Entomogenous Protozoa and Fungi (C.M. Ignoffo.Ed.), Boca Raton: CRC. Vol. 5. Pp. 151-236.

McCreight, J. D., Herson, H. and Grumet, R. 2016. Melon. In: Kalloo G and Bergh BO (eds) Genetic Improvement of Vegetable Crops. pp 267-294. Pergamon Press. Oxford, England.

McGregor, C. E. and Waters, V. 2014. Flowering patterns of pollenizer and triploid watermelon cultivars. *HortScience* 49.6:714-721.

MedicineNet, 2004. Definition of Watermelon. Retrieved Sept. 26, 2019 from http://www.medicinenet.com/script/main/hp.asp.

Mehinto, J. T., Atachi, P., Douro Kpindou, O. K., Dannon, E. A. and Tamò, M. 2014. Mortality of *Maruca vitrata* (Lepidoptera: Crambidae) larval stages induced by different doses of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana. International Journal of Advanced Research* 2.4:273-285.

Mercy, G. A. and Bosa, E. O. 2013. The morphological characterization of the melon species in the family cucurbitaceae. juss. and their utilization in Nigeria. *International Journal of Modern Botany* 3.2:15-19

Mergenthaler, M., Weinberger, K. and Qaim, M. 2009. The role of consumers' perceptions in the valuation of food safety and convenience attributes of vegetables in Vietnam, pp. 1-20.

Meyling, N. V. and Eilenberg, J. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control* 43:145-155.

Minas, R. D. S., Souza, R. M., Dolinski, C., Carvalho, R. D. S. and Burla, R. D. S. 2016. Potential of entomopathogenic nematodes (Rhabditida: Heterorhabditidae) to control Mediterranean fruit fly (Diptera: Tephritidae) soil stages. *Nematoda* 3.

Minhibo, M. Y., Ndepo, O. R., Hala, N., Koua, H., Tuo, Y., N'Goran, A., Adama Coulibaly, A., Doumbia, S., Djaha, J-B. A., Kouakou, C. K., Adopo, A. N., Nda, H. A. and Djidji, H. 2018. Assessment of Fruit Fly Trapping System in Mango Orchards in Northern Côte d'Ivoire. J*ournal of Agricultural Science and Technology* 18-27.

Mkiga, A. M.; Mwatawala, M. W. Developmental Biology of *Zeugodacus cucurbitae* (Diptera: Tephritidae) in Three Cucurbitaceous Hosts at Different Temperature Regimes. *Journal of Insect Science* 15:160.

Mohammadbeigi, A, Port, G. 2013 Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against Uvarovistia zebra (Orthoptera: Tettigoniidae) via contact and ingestion. *International Journal of Agricultural Crop Science* 5.2:138-146.

Moore, D., Bridge, P. D., Higgins, P. M., Bateman, R. P., Prior, C. 1993. Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Annals of Applied Biology* 122.3: 605–616.

Muhammad, L. and Kiilu, F. M. 2004. Reducing risk by fruit processing. LEISA Magazine,*http://www.agriculturesnetwork.org/magazines/global/from-field-to-market/reducingrisk-byfruit-processing*

Muñoz, R. J. A. 2000. Pathogenicity of *Beauveria bassiana* (Bals.) Bullí. on the Mediterranean fly, *Ceratitis capitata* (Wied.) under laboratory conditions. Bachelor Thesis Faculty of Agricultural Sciences. Autonomous University of Cchiapas. Huehuetán, Chis., Mexico. 55 p

Mwatawala, M., Maerere, A. P., Makundib, R. and de Meyer, M., 2010. Incidence and host range of the melon fruit fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in Central Tanzania. *International Journal of Pest Management* 56.3:265-273.

Mwatawala, M., Kudra, A., Mkiga, A., Godfrey, E., Jeremiah, S., Virgilio, M. and de Meyer, M. 2015. Preference of *Zeugodacus cucurbitae* (Coquillett) for three commercial fruit vegetable hosts in natural and semi natural conditions. *Fruits* 70.6:333-339.

Naaz, N., Choudhary, J., Prabhakar, S., Moanaro, C. S. and Maurya, S. 2016. Identification and evaluation of cultivable gut bacteria associated with peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae). *Phytoparasitica* 44:165-176.

Nagamani, A., Kunwar, I. K. and Manoharachary, C. 2006. Hand book of soil fungi. IK International pvt, ltd. New Delhi. 1-51.

Niassy, S., Maniania, N. K., Subramanian, S., Gitonga, L. M. and Ekesi, S. 2012. Performance of a semiochemical-baited autoinoculation device treated with Metarhizium anisopliae for control of Frankliniella occidentalis on French bean in field cages. *Entomologia Experimentalis et Applicata* 142.2:97-103.

Norrbom, A. 2004. Fruit fly (Diptera: Tephritidae) Classification and diversity Systematic and entomology. Laboratory, ARS, USDA Department of entomology.

Norrbom, A. L., Caroll, L. E., Thompson, R. C., White, I. M. and Freidberg, A. 1999. Systematic database of names. In: Thompson FC. (Ed.) Fruit Fly Expert Identification System and Systematic Information Database. *Myia* 9:65-251.

Ogwu, M. C., Osawaru, M. E. and Aiwansoba, R. O. 2016. Comparative Assessment of Some Physical Characteristics of Watermelon (*Citrullus lanatus*). Proc. NTBA/NSCB Joint Biodiversity Conference, Unilorin, pp. 41-46.

Oliveira, C. M., Auad, A. M., Mendes, S. M and Frizzas, M. R. 2013. Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology* 137:1-15.

Omoloye, A. A., Ajifolokun, A. O. and Tobih, F. O. 2015. Sources and abundance of fungi with entomopathogenic potential for control of the cowpea pod borer, *Maruca vitrata* Fab. in Ibadan, Nigeria. *Journal of Entomology and Nematology* 7.3:18-25.

Paris, H. 2017. Origin of the dessert watermelon, *Citrullus lanatus. Acta Horticulturae*. 1151:87-94.

Patel, R. K. and Patel, C. B. 1998. Biology of fruit fly, *Dacus ciliatus* Loew (Tephritidae: Diptera) infesting little gourd, *Coccinia indica* W. and A. *Gujarat Agricultural University Research Journal* 23.2:54-60.

Peters, A. 1997. Prospects for the use of biological control agents to control fruit flies, pp. 199-204. In *Management of Fruit Flies in the Pacific.* Regional symposium. 28-31 October 1996, Nadi, Fiji (edited by Allwood, A. J. and Drew, R. A. I.). ACIAR Proceedings No. 76.

Phophi, M. M. and Mafongoya, P. L. 2017. Constraints to Vegetable Production Resulting from Pest and Diseases Induced by Climate Change and Globalization: A Review. *Journal of Agricultural Science* 9:10-11.

Pierre, D. J., Quilici, S., Ratnadass, A., Ryckewart, P., and Vayssières, J. F. 2015. "Fruit Flies (Diptera: Tephritidae) Identified in the Southern Part of Haiti." *Faunistic Entomology* 68:193-200.

Potrich, M. Dallacort, S., Luckman, D., Lozano, E. R., Pegorini, C. S. and Simionatto, D. 2018. Compatibility of *Beauveria bassiana* and alternative phytosanitary products. J*ournal of Applied Microbiology* 125.6:1802-1811.

Prior, C., Carey, M., Abraham, Y. T., Moore, D. and Bateman, R. P. 1995. Development of a bioassay method for the selection of entomopathogenic fungi virulent to the desert locust *Schistocerca gregaria* (Forskål). *Journal of Applied Entomology* 119:567-573.

Qazzaz, F. O., Al-masri, M. I. and Barakat, R. M. 2015. Effectiveness of *Beauveria bassiana* native isolates in the biological control of the Mediterranean fruit fly (*Ceratitis capitata*). *Advances in Entomology* 3:44-55.

Quesada-Moraga, E., Ruiz-García, A. and Santiago-Álvarez, C. 2006. Laboratory Evaluation of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* Against Puparia and Adults of *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Economic Entomology* 99.6:1955-1966.

_____, Santos-Quirós, R., Valverde-García, P. and Santiago-Álvarez, C. 2004. Virulence, horizontal transmission, and sub-lethal reproductive effects of *Metarhizium anisopliae* (Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). *Journal of Invertebrate Pathology* 87:51-58.

Qin, Y., Paini, D. R., Wang, C., Fang, Y. and Li, Z. 2015. Global establishment risk of economically important fruit fly species (Tephritidae). PloS one 10:1.

Radić, S., Stipaničev, D., Vujčić, V., Rajčić, M. M., Širac, S. and Pevalek-Kozlina, B. 2010. The evaluation of surface and waste water genotoxicity using the *Allium cepa* test. *Science of The Total Environment* 408.5:1228-1233.

Rai, D., Updhyay, V., Mehra, P., Rana, M. and Pandey, A. K. 2014. Potential of entomopathogenic fungi as biopesticides. *Indian Journal of Science Research and Technology* 2.5:7-13.

Raisbeck, D. W. 2003. The role of agriculture in the Global Economy. In *World Agricultural Forum, St. Louis, Mo*.

Reganold, J. P. and Wachter, J. M. 2016. Organic agriculture in the twenty-first century. *Nature plants 2*.2:15-22.

Renner, S. S. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. Retrieved Sept. 23, 2019, from https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.13163

_____.and Schaefer, H. 2016. "Phylogeny and evolution of the Cucurbitaceae". In Grumet R, Katzir N, Garcia-Mas J (eds.). Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models. 20. New York, NY: Springer International Publishing. pp. 1-11.

Rizvi, R., Ansari, R. A., Safiuddin Agrawal, P., Sumbul, A., Tiyagi, S. A. and Mahmood, I., 2015. Effect of some organic fertilizers and bioinoculant on growth attributes of tomato in relation to sustainable management of root-knot nematode. *Journal of Plant Pathology* 115:206-215.

Reynolds, O. L., Osborne,T. J. and Barchia, I. 2018. Efficacy of Chemicals for the Potential Management of the Queensland Fruit Fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Journal of Insect Science* 18.4:16.

Roberts, D. W. and Hajek, A. E. 1992. Entomopathogenic fungi as bioinsecticides. In *Frontiers in industrial mycology* (pp. 144-159). Springer, Boston, MA.

Ronald, F. l. M. and Kessing, J. M. 2007. *Bactrocera cucurbitae* (Coq.). Retrieved Sept. 15, 2019 from http//www.extento.hawaii.edu/kbase/crop/Type/bactro_c.htm.

Rouse, S., Harnett, D., Vaughan, A. and Van Sinderen, D., 2008. Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *Journal of Applied Microbiology* 104:915-923.

Roy, S. W. and Penny, D. 2006. Smoke without fire: most reported cases of intron gain in nematodes instead reflect intron losses. *Molecular biology and evolution* 23.12:2259-2262.

Ruiu, L. 2015. Insect pathogenic bacteria in integrated pest management. *Insects* 6.2:352-367.

Samson, R. A. and Evans, H. C. 1982. Two new *Beauveria* spp. from South America. *Journal of Invertebrate Pathology* 39.1:93-97.

Salehi, B., Capanoglu, E., Adrar, N., Catalkaya, G., Shaheen, S., Jaffer, M. and Docea, A. O. 2019. Cucurbits plants: A key emphasis to its pharmacological potential. *Molecules* 24:10-18.

Sapkota, R. K. Dahal, C. and Thapa, R. B. 2010. Damage assessment and management of cucurbit fruit flies in spring-summer squash. *International Journal of Nematology and Entomology* 1.4:064-068.

Sapna, J. N., Bai1, O. K. Remadevi, T., Sasidharan, O., Balachander, M., Priyadarsanan, D. 2012. Cuticle degrading enzyme production by some isolates of the entomopathogenic fungus, *Metarhizium anisopliae* (metsch.). *Journal bio-science* 20:25-32.

Sarwar, M. 2015. Cultural Measures as Management Option Against Fruit Flies Pest (Tephritidae: Diptera) in Garden or Farm and Territories. I*nternational Journal of Animal Biology* 1.5:165-171.

_____, Ahmad, N., Rashid, A. and Shah, S. M. M. 2015. Valuation of gamma irradiation for proficient production of parasitoids (Hymenoptera: Chalcididae and Eucoilidae) in the management of the peach fruit-fly, *Bactrocera zonata* (Saunders). *International Journal of Pest Management* 61.2:126-13.

Schippers, R. R. 2004. Légumes africains indigènes. Présentation des espèces cultivées. Margrafs Publishers GmbH, Scientific Books, 2004. 482 p.

_____. 2000. African Indigenous Vegetable. pp. 56-60. An Overview of the Cultivated species. Chatthan, U.K., N.R/ACO. EU.

Scholte, E. J., Knols, B. G., Samson, R. A. and Takken, W. 2004. Entomopathogenic fungi for mosquito control: a review. *Journal of insect science*, 4.1.

_____, Takken, W. and Knols, B. G. J. 2003a. Pathogenicity of five African entomopathogenic fungi against *Anopheles gambiae s.s.* 156 mosquitoes (Diptera: Culicidae). *Proceeding of the Experimental and Applied Entomology of the Netherlands Entomological Society* 14:25-29.

Shah, S. M. M., Ahmad, N., Sarwar, M. and Tofique, M. 2014. Rearing of *Bactrocera zonata* (Diptera: Tephritidae) for parasitoids production and managing techniques for fruit flies in mango orchards. *International Journal of Tropical Insect Science* 34.1:108-113.

Sinzogan, A., Van Mele, P. and Vayssières, J-F. 2008. Implications of on-farm research for local knowledge related to fruit flies and the weaver ant, *Oecophylla longinoda* in mango production. *International Journal of Pest Management* 54.3:241-246.

Sookar, P., Bhagwant, S. and Awuor Ouna, E. 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). *Journal of Applied Entomology* 132.9-10:778-788.

St Leger, R. J., Roberts, D. W. and Staples, R. C. 1991. A model to explain differentiation of appressoria by germlings of *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* 57.3:299-310.

Stark, J. D., Vargas, R. I., Souder, S. L., Fox, A. J., Smith, T. R. and Mackey, B. R. U. C. E. 2013. A comparison of the bioinsecticide, spinosad, the semi-synthetic insecticide, spinetoram and synthetic insecticides as soil drenches for control of tephritid fruit flies. *Biopesticide International* 9:120-126.

Strasser, H., Abendstein, D. and Stuppner, H. 2000. Monitoring the distribution of secondary metabolites produced by the entomopathogenous fungus *Beauveria brongniartii* with particular reference to oosporein. *Mycological Research* 104.10:1227-1233.

Suckling, D. M., Kean, J. M., Stringer, L. D., Caceres-Barrios, C., Hendrichs, J., Reyes-Flores, J. and Dominiak, B. C. 2014. Eradication of tephritid fruit fly pest populations: outcomes and prospects. Pest Management Science.

Sujeetha, J. A. R. and Sahayaraj, K. 2014. Role of entomopathogenic fungus in pest management. In *Basic and Applied Aspects of Biopesticides* (pp. 31-46). Springer, New Delhi.

Sun, J., Fuxa, J. R. and Henderson, G. 2003. Virulence and in vitro characteristics of pathogenic fungi isolated from soil by baiting with *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Journal of Entomological Science* 38.3:342-358.

Tan, K. H., Nishida, R., Jang, E. B. and Shelly, T. E. 2014. Pheromones, male lures, and trapping of tephritid fruit flies. In *Trapping and the detection, control, and regulation of tephritid fruit flies* (pp. 15-74). Springer, Dordrecht.

Tatlioglu, G. 1993. Cucumber. In: Kalloo G and Bergh BO (eds) Genetic Improvement of Vegetable Crops. pp 197-234. Pergamon Press. Oxford, England

Thaochan, N. N. and Ngampongsai, A. 2018. Effect of *Metarhizium guizhouense* infection on mating competition and mate choice of *Bactrocera latifrons* (Diptera: Tephritidae). *Phytoparasitica* 46.4:459-469.

This Day Live, 2014. How Export Farm Produce will Improve Nigeria‟s Foreign Earnings. Media Report. Retrieved May 22, 2018 from www.thisdaylive.com/articles/how.

Toledo, J., Flores, S., Campos, S., Villaseñor, A., Enkerlin, W., Liedo, P. and Montoya, P. 2017. Pathogenicity of three formulations of *Beauveria bassiana* and efficacy of autoinoculation devices and sterile fruit fly males for dissemination of conidia for the control of *Ceratitis capitata*. *Entomologia Experimentalis et Applicata* 164.3:340-349.

Uziel, 2003. Infection of *Ceratitis capitata* by Two Species of the *Entomophthora muscae* Species Complex (Zygomycetes: Entomophthorales) in the Field. *Phytoparasitica* 31.2:204-206

van Bruggen, A. H., Gamliel, A. and Finckh, M. R. 2016. Plant disease management in organic farming systems. *Pest management science 72.*1:30-44.

Van Mele, P. 2008. A historical review of research on teh weaver ant *Oecophylla* in biological control. *Agricultural and Forest Entomology* 10:13-22.

Vargas, R. I., Walsh, W. A., Kanehisa, D., Stark, J. D. and Nishida, T. 2000. Comparative demography of three Hawaiian fruit flies (Diptera: Tephritidae) at alternating temperatures. *Annals of the Entomological Society of America* 93.1:75-81.

Vargas, R. I., Mau, R. F. L., Jang, E. B. and Wong, L. 2010. Area-wide fruit fly integrated pest *of Economic Importance* (eds Sabater-Munoz, B., Llopis, V.N. and Garcia, A.U.), pp. 171-178. Editorial Universitat Politecnica de Valencia, Valencia, Spain.

_____, Piñero, J. C. and Leblanc, L. 2015. An Overview of Pest Species of *Bactrocera* Fruit Flies (Diptera: Tephritidae) and the Integration of Biopesticides with Other Biological Approaches for Their Management with a Focus on the Pacific Region. *Insects* 6:297-318.

_____, Souder, S. K., MacKey, B., Cook, P., Morse, J. G. and Stark, J. D. 2012. Field trials of solid triple lure (trimedlure, methyl eugenol, raspberry ketone, and DDVP) dispensers for detection and male annihilation of *Ceratitis capitata*, *Bactrocera dorsalis* and *Bactrocera Cucurbita*(Diptera: Tephritidae) in Hawaii. *Journal of economic entomology* 105.5:1557-1565.

Vasudha, A. and Agarwal, M. L. 2019. Management of Dacine fruit flies (Tephritidae: Dacinae: Dacini) in horticultural ecosystems: A review. *Journal of Entomology and Zoology Studies* 7.3:33-42.

Vayssières, J. F., Goergen, G., Lokossou, O., Dossa, P. and Akponon, C. 2005. A new *Bactrocera* species in Benin among mango fruit fly (Diptera: Tephritidae) species. *Fruits* 60.6:371-377.

_____, Rey, J-Y. and Traoré, L. 2007. Distribution and host plants of *Bactrocera cucurbitae* in West and Central Africa. *Fruits* 62.6:391-396.

_____, Sinzogan, A. and Bokonon-Ganta, A. H. 2008. La nouvelle espèce invasive de mouches des fruits: *Bactrocera invadens* Drew Tsuruta et White. Consulté en 2010, sur Projet Régional de Lutte Contre les Mouches des Fruits en Afrique de l'Ouest. *http://www.cirad.bf/fr/anx/mouche1 02.php*

_____, Sinzogan, A. A. C., Adandonon, A., Van Mele, P., Korie, S. 2013. Ovipositional behaviour of two mango fruit fly species (Diptera, Tephritidae) in relation to Oecophylla cues (Hymenoptera, Formicidae) as compared to natural

conditions without ant cues. *International Journal Biological Chemistry Science* 7:447-456.

Ventura, C., Briones-Roblero, C. I., Briones-Roblero, E., Rivera-Orduña, F. N. and Zúñiga, G. 2018. Comparative analysis of the gut bacterial community of four Anastrepha fruit flies (Diptera: Tephritidae) based on pyrosequencing. *Current Microbiology* 75.8: 966-976.

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A. and Keller, S. 2009. Fungal entomopathogens: New insights on their ecology. *Fungal Ecology* 2:149-159.

Virgilio, M., Jordaens., K., Verwimp, C., White, I. M. and de Meyer, M. 2015. Higher phylogeny of frugivorous flies (Diptera: Tephritidae, Dacini): Localised partition conflicts and a novel generic classification, Molecular Phylogenetics and Evolution.

Waksman, S. A.1994. Three decades with soil fungi. *Soil science* 58:89-114.

Wang, Y. H., Behera, T. K. and Kole, C. (Eds.). 2011. Genetics, genomics and breeding of cucurbits. CRC Press.

Weems, Jr., H. V., Heppner, J. B., Fasulo, T. R. and Nation, J. L. 2001. Caribbean Fruit Fly, *Anastrepha suspensa* (Loew) (Insecta: Diptera: Tephritidae). EENY-196, Featured Creatures from the Entomology and Nematology Department, Florida Cooperative Extension Service, Insitute of Food and Agricultural Sciences. University of Florida.

_____. and Fasulo, L. M. 2002. Apple Maggot, Apple Maggot Fly, *Rhagoletis pomonella* (Walsh) (Insecta: Diptera: Tephritidae). University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

Wehner, T. C, Shetty, N. V. and Elmstrom, G. W. 2001. Breeding and seed production in Watermelons. Characteristics, production, and marketing (Maynard DN, ed), ASHS Press, Alexandria (VA, USA) p. 27-73.

White, I. M., de Meyer, M. and Stonehouse, J. 2001. A review of the native and introduced fruit flies (Diptera, Tephritidae) in the Indian Ocean Islands of Mauritius, Réunion, Rodrigues and Seychelles. In: Price NS, Seewooruthun I. (Eds) Proceedings of the Indian Ocean Commission Regional Fruit Fly Symposium, Mauritius, 5-9th June 2000. Indian Ocean Commission, Mauritius, 15-21.

_____. 2006. Taxonomy of the Dacina (Diptera: Tephritidae) of Africa and the Middle East. A*frican Entomology Memoir* 2:1-156. Retrieved Jul. 15, 2017 from http://journals.sabinet.co.za/essa

_____, and Elson-Harris, M. M. 1992. Fruit flies of economic significance: their identification and bionomics pp. 601 London: CAB International, Wallingford, UK.

World bank, 2016. The World Bank Annual Report 2016. Washington, Retrieved Jul. 12, 2019 from https://openknowledge.worldbank.org/handle/10986/24985.

_____, 2019. Agriculture, forestry and fishing value added (% of Gross Domestic Product. Retrieved Oct. 17, 2019, from, https://data.worldbank.org/indicator/NV.AGR.TOTL.ZS?end=2018&locations=BJ&most_recent_year_desc=false&start=2006&view=chart Wraight, S. P., Jacksonz, M. A. and De Kock, S. L. 2001. Formulation of Fungal Biocontrol Agents. *Fungi as biocontrol agents: Progress problems and potential*, *253*.

Wraight, S. P., Jacksonz, M. A. and De Kock, S. L. 2001. Formulation of Fungal Biocontrol Agents. Fungi as biocontrol agents: Progress problems and potential, 253.

Www.planfor.fr/achat pastèque, Retrieved Oct. 23, 2019, from, htttps://www. planfor.fr/achat pastèque,

Yang, Y., Zhang, Y., Wang, M., Li, S. S., Ma, X. Y. and Xu, Z. H. 2015. Bioefficacy of entomopathogenic *Aspergillus* strains against the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). *Applied Entomology and Zoology 50.*4:443-449.

Yeo, H., Pell, J. K., Alderson, P. G., Clark, S. J., Pye, B. J. 2003. Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species. *Pest Management Science* 59.2: 156–165.

Yong, H. S., Song, S. L., Chua, K. O. and Lim, P. E. 2017a. Microbiota associated with *Bactrocera carambolae* and *B. dorsalis* (Insecta: Tephritidae) revealed by next-generation sequencing of 16S rRNA gene. *Meta Gene* 11:189-196.

Yuan, S. Y., Kong, Q., Tian, X. J., Guo, Y. L, Chen, B. and Li, X. 2013. Study on Laboratory toxicity of *Beauveria bassiana* strain to *Bactrocera cucurbitae* Coquillett. *Northern Horticulture* 7:137-140.

Zhao, X., Zhang, X., Chen, Z., Wang, Z., Lu, Y. and Cheng, D. 2018. The divergence in bacterial components associated with *Bactrocera dorsalis* across developmental stages. *Frontiers in Microbiology* 9.114.

Zhou, X. W., Niu, C. Y., Han, P. and Desneux, N. 2012. Field evaluation of attractive lures for the fruit fly *Bactrocera minax* (Diptera: Tephritidae) and their potential use in spot sprays in Hubei Province (China). *Journal of Economic Entomology* 105.4:1277-1284.

Zimmermann, G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. *Journal of Applied Entomology* 102:213-215.

_____. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology* 17:553-596.