EVALUATION OF THE INSECTICIDAL ACTIVITY OF CASHEW (*Anacardium occidentale* L.) NUT SHELL LIQUID AGAINST ADULT *Bactrocera dorsalis* (HENDEL) (DIPTERA: TEPHRITIDAE)

SAINEY KEITA

UNIVERSITI SAINS MALAYSIA

2022

EVALUATION OF THE INSECTICIDAL ACTIVITY OF CASHEW (*Anacardium occidentale* L.) NUT SHELL LIQUID AGAINST ADULT *Bactrocera dorsalis* (Hendel) (DIPTERA: TEPHRITIDAE)

by

SAINEY KEITA

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

October 2022

ACKNOWLEDGEMENT

I wish to express my profound gratitude to ALLAH SWT for giving me the strength and health to carry out this study. A very special thanks to my supervisor Associate Professor Ts Dr Wan Fatma Zuharah for her guidance throughout my journey, her patience and kindness has been my inspiration throughout my work. I also wish to express my sincere gratitude to my wife Fatma who shouldered the burden of taking care of the family during these long years. To my kids Mohammed, Mariatou, Yaya, Yai and Teneng for being able to cope with all the challenges they faced in their day- to-day tasks during my absence. My profound thanks to brothers and sisters who stepped for me and took great of my family when the need arose. My sincere thanks go to my friends and colleagues of the Medical Entomology laboratory of the Vector Control Research Unit, USM for the cordial relationship accorded me and all the fruitful discussions we had which help to shape my work. More specially to Mr Muthu who has been driving me to and from the field for all my field work. A special thanks to the management and staff of Agricultural farm Ara Kuda for allowing me to conduct my field experiments on their farm. I thank most profoundly the Islamic Development Bank (IDB) through the Government of The Gambia (GoTG) for sponsoring me and the University of The Gambia Management for nominating me for this program. I also wish to thank my fellow students both local and international for providing me the comfort at times when both spirit and body were failing. Finally, I dedicate this work to my late parents Alhagi Kalifa Keita and Ajaratou Maimuna Jallow. May their Souls continue to Rest in Peace Ameen.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS

√	Square root
µL	Micro Litre
a.i	Active ingredient
nm	Nano meters
P	Polarity index
ppm	parts per million
r	Pearson regression coefficient
v/v	Volume by volume

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ANOVA	Analysis of Variance
COI	Cytochrome oxidase I
CNSL	Cashew Nut Shell Liquid
CRD	Completely Randomized Design
DDT	Dichlorodiphenyltrichloroethane
FAOSTAT	Food and Agriculture Statistics
FAW	Fall Armyworm
GC-MS	Gas Chromatography Mass Spectrophotometer
MANOVA	Multivariate Analysis of Variance
ME	Methyl Eugenol
SIT	Sterile Insect Technique
TEPP	Tetraethyl pyrophosphate
IPM	Integrated Pest Management

LIST OF APPENDICES

PENILAIAN AKTIVITI INSEKTISID CECAIR KULIT KACANG GAJUS *(Anacardium occidentale* L.) TERHADAP *Bactrocera dorsalis* (HENDEL) (DIPTERA: TEPHRITIDAE) DEWASA

ABSTRAK

Lalat buah Oriental, *Bactrocera dorsalis* (Hendel), adalah salah satu daripada perosak buah-buahan pertanian yang paling terkenal. Ia didapati menyerang lebih 250 tumbuhan perumah, termasuk buah-buahan komersial dan pelbagai jenis tanaman. Aktiviti insektisid gajus (*Anacardium occidentale* L.) cecair kulit kacang (CNSL) terhadap *Bactrocera dorsalis* (Hendel) dewasa telah disiasat di bawah keadaan makmal dan lapangan. Dua pelarut pengekstrakan, diklorometana dan heksana telah digunakan untuk mengekstrak CNSL daripada kulit kacang gajus. Ketoksikan sentuhan, penggunaan topikal dan ujian penolakan telah dijalankan di makmal. Untuk kedua-dua eksperimen sentuhan dan topikal, kepekatan 0.625, 1.25, 2.5, 5.0, 10.0 dan 20.0 x 104 ppm telah disediakan, manakala 0.07, 0.14, 0.21, 0.28 dan 0.35 µL/cm2 telah disediakan untuk ujian penolakan. Nilai LC_{50} yang direkodkan selepas 24 jam pendedahan untuk ujian ketoksikan sentuhan ekstrak diklorometana ialah 4.66 x 10^4 ppm, manakala 6.58 x 10^4 ppm direkodkan untuk ekstrak heksana, yang mana menunjukkan ekstrak diklorometana adalah lebih tinggi secara signifikan dengan 1.32 kali lebih maut daripada ekstrak heksana terhadap *B. dorsalis* (ujian-t, $P<0.05$). Eksperimen penggunaan topikal mencatatkan 100% kematian untuk ekstrak diklorometana, manakala 82.5% kematian telah direkodkan untuk ekstrak heksana selepas 24 jam pendedahan. Ujian-T bebas yang dijalankan secara berasingan pada nilai-nilai ini untuk ujian aplikasi topikal menunjukkan bahawa angka kematian yang lebih tinggi didaftarkan daripada ekstrak diklorometana daripada heksana (ujian-t,

$P<0.05$). Ujian penolakan menunjukkan ekstrak heksana mempunyai tindakan penghalau yang lebih kuat terhadap *B. dorsalis*. Pada kepekatan 0.35 µL/cm2, ekstrak heksana mencatatkan penolakan sebanyak 76%, manakala ekstrak diklorometana mencatatkan penolakan sebanyak 48% pada kepekatan yang sama selepas 24 jam. Analisis GC-MS telah mengesan kehadiran Fenol, 3-pentadecil- yang dikesan dengan kepekatan yang lebih tinggi iaitu 95.5% dalam ekstrak diklorometana CNSL dan hanya 46.75% dalam ekstrak heksana. Dalam kajian makmal kami menggunakan botol yang dicat dengan warna kuning, hijau kekuningan atau hijau, kami mendapati tiada pemilihan warna diperhatikan untuk ujian lalat tunggal ($p>0.05$). Manakala pemilihan warna diperhatikan dalam ujian dengan lima lalat ($p<0.05$). Dalam kajian penilaian lapangan, faktor persekitaran mempengaruhi tingkah laku lalat dengan menunjukkan hubungan yang signifikan dengan bilangan lalat jantan dan lalat betina yang terperangkap. Bilangan jantan yang ditangkap adalah dipengaruhi oleh hujan secara lemah ($r=0.152$, $P<0.05$) dan kelajuan angin bagi latat betina ($r=0.167$, $P<0.05$). Kesan warna yang digabungkan dengan umpan dan penarik (metil eugenol) disediakan daripada ekstrak diklorometana menyebabkan lebih banyak kematian kepada *B. dorsalis* (ANOVA Faktorial, $p<0.05$). Menggunakan perangkap Steiner yang diubahsuai dan dicat dengan warna kuning, hijau kekuningan atau hijau menunjukkan perangkap kuning menarik lebih banyak *B. dorsalis* walaupun tiada perbezaan signifikan yang diperhatikan. Oleh itu, keputusan kami telah membuktikan bahawa ekstrak CNSL diklorometana boleh bertindak sebagai pengganti kawalan untuk pengurusan *B. dorsalis*. Ini menjadikannya sumber alternatif yang ideal kerana kemunculan kerintangan oleh *B. dorsalis* terhadap beberapa racun serangga sintetik.

EVALUATION OF THE INSECTICIDAL ACTIVITY OF CASHEW (*Anacardium occidentale* L.) NUT SHELL LIQUID AGAINST ADULT *Bactrocera dorsalis* (HENDEL) (DIPTERA: TEPHRITIDAE)

ABSTRACT

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is one of the most notorious pests of agricultural fruits. It has been found to attack over 250 host plants, including commercial fruits and a wide variety of crops. The insecticidal activity of Cashew (*Anacardium occidentale* L.) nut shell liquid (CNSL) against adult *B. dorsalis* (Hendel) was investigated under laboratory and field conditions. Two extracting solvents, dichloromethane and hexane were used to extract the CNSL from the cashew nut shell. Contact toxicity, topical application and repellency tests were carried out in the laboratory. For both the contact and topical experiments, concentrations of 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 x 10^4 ppm were prepared, whereas 0.07, 0.14, 0.21, 0.28 and 0.35 µL/cm^2 concentrations were prepared for the repellency tests. The LC_{50} values recorded after 24 hours of exposure for the contact toxicity tests of the dichloromethane extract was 4.66 x 10^4 ppm, whereas 6.58 x 10^4 ppm was recorded for the hexane extract, which showed the dichloromethane extract to be significantly higher with 1.32 times more lethal than the hexane extract against *B. dorsalis* (t-test, $P<0.05$). The topical application experiments showed100 % mortality for the dichloromethane extract, whereas 82.5% mortality was recorded for the hexane extract after 24 hours of exposure. An independent T-test carried out separately on these values for the topical application assay showed higher mortality figures registered from dichloromethane extracts than hexane (t-test, $P<0.05$). The repellency tests showed the hexane extract had a stronger repellent action against *B.*

dorsalis. At a concentration of 0.35 µL/cm^2, the hexane extract recorded a repellency of 76%, whereas the dichloromethane extract registered 48% repellency at the same concentration after 24 hours. Gas Chromatography-Mass Spectrophotometer detected presence of phenol, 3-pentadecyl- with a higher concentration of 95.5% in CNSL dichloromethane extract and only 46.75% in hexane extract. In our laboratory study using bottles painted with yellow, yellowish-green or green colours we found that no colour preference was observed for the single fly test ($p>0.05$). Whereas colour preference was observed with the five flies test ($p<0.05$). In the field evaluation study, environmental factors influenced the behaviour of the flies by showing a significant relationship with the number of male and female flies trapped. The number of males captured was weakly influenced by rainfall ($r=0.152$, $P<0.05$) and windspeed for female flies ($r=0.167$, $P<0.05$). The effect of colour combined with baits and attractant (methyl eugenol) prepared from dichloromethane extract of CNSL caused more mortality to *B. dorsalis* (Factorial ANOVA, $p<0.05$). Using modified Steiner traps painted with yellow, yellowish-green or green colours showed that yellow traps attract more *B. dorsalis* even though no significant difference were observed. Thus, our results have proven that the dichloromethane CNSL extract could act as a control substitute for the management of *B. dorsalis.* This makes it an ideal alternative source due to the emergence of resistance by *B. dorsalis* to some synthetic insecticides.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The Dipteran family Tephritidae, to which the papaya fruit flies *Bactrocera dorsalis* (Hendel) belong, consists of over 4500 known species. They are considered to be the world's most significant agricultural pests (Daane & Johnson, 2010). Their prevalence in all the regions of the world makes them to be a very important pest. Thus, an important threat to horticultural crops which include papaya (Singh & Sharma, 2013). It is a highly invasive pest of a broad range of agricultural fruit crops. Because of its destructive nature, it has caused significant direct losses in yields and indirect losses due to quarantine restriction on potentially infested fruit resulting in diminished market access across the globe (Toshiyuki Dohino et al., 2016).

Carica papaya L., described by Linnaeus, (1753), belongs to the family Caricaceae and is commonly referred as papaya and is believed to have originated from tropical America (Yogiraj et al., 2014; Fuentes & Santamaría, 2014). Papaya is an economically important crop with an annual global production figure of 13,016,281 tons (Daagema et al., 2020) and a gross global value of 3.5 billion USD (Fuentes & Santamaría, 2014). Hence this makes *B. dorsalis* a major pest of papaya an important pest that needs serious attention to reduce the losses experienced by the papaya industry globally.

Currently, management options of *B. dorsalis* include chemical insecticide application (Vontas et al., 2011). For example, in China control of *B. dorsalis* relies almost exclusively on insecticides rather than other integrated techniques (Jin et al., 2011). Thus, resistance to synthetic insecticides by *B. dorsalis* have been reported in

Hawaii (Chou et al., 2010) and mainland China (Jin et al., 2011). Other control strategies of the pest include strict quarantine efforts (B. Liu et al., 2017) and the application of plant-derived insecticides (Hikal et al., 2017).

It is estimated that there are over 100,000 secondary plant metabolites and out of these, over hundreds metabolites have demonstrated bioactivity against insects which can be categorized as behavioural or physiological (Isman, 2017). These metabolites are now used as botanical insecticides against a wide variety of insect pests. They have the beauty of being compatible with organic farming systems and have multiple modes of action, retarding the ability of insects to develop resistance (Walad et al., 2015).

Cashew nut shells (CNS), is an agricultural wastes from cashew nut processing factories (Hamad & Mubofu, 2015). The cashew nut shell liquid (CNSL) extracted from the cashew nut shell is a promising alternative to chemical insecticides because several studies that used substances extracted from the cashew nuts shell have been found to possess insecticidal properties (Carvalho et al., 2019). Hence this investigation on the insecticidal and repellent activity of CNSL against *B. dorsalis* is aimed at contribution to this missing link in insect pest control, thus in a way filling this void.

There are 134 species of arthropods that affect papaya. Most of the species are insects, while 12 belong to the Acarina. Twenty-six out of these arthropod species are fruit flies in the family Tephritidae (Peña et al., 2002). *Bactrocera dorsalis* like all other fruit flies are a serious pest on horticultural crops including papaya throughout the tropical and subtropical regions. They destroy crops by laying eggs under the skin of their host. The eggs hatch into larvae feeding in the decaying tissues which quickly become rotten and inedible or drop to the ground prematurely, thus causing considerable losses in production (Hasyim et al., 2016).

The use of pesticidal plants to control pests has been in existence since ancient times. They were used extensively until the 1940 when synthetic insecticides were introduced (Khater, 2012). They are mentioned in Hieroglyph, Chinese, Greek, and Roman antiquity and India, the use of the neem tree (*Azadirachta indica Juss*.; Meliaceae) was reported in the Veda, a body of manuscripts written in archaic Sanskrit dated at least four millennia ago (Philogène et al., 2005). In modern Europe and North America, records show that the use of botanicals extends back for more than 150 years, predating the introduction of the major classes of synthetic chemical pesticides (organochlorines, organophosphates, carbamates, and pyrethroids) in the mid-1930s to 1950s because of its indiscriminate use have caused many environmental problems such as pollution and insect resistances (El-Wakeil, 2013).

A study on the anti-insect activity of Cashew Nut Shell Liquid (CNSL) as a botanical insecticide therefore harmoniously fits in this crusade of finding alternatives to synthetic pesticides to produce healthy food and in the process save the environment from toxic chemicals that are present in synthetic pesticides.

The cashew nut shell (CNS) is released into the environment as an agricultural by product and waste. However, inside the soft honeycomb of the shell, there is a valuable viscous liquid called cashew nut shell liquid (Hamad & Mubofu, 2015). Cashew Nut Shell Liquid (CNSL) is a unique source of naturally occurring long-chain hydrocarbon phenols and constitutes about 25% of the cashew weight and 30%–35% of the nut shell weight (Mazzetto et al., 2009). Typically, solvent-extracted

CNSL contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%), and traces of 2-methyl cardol. (Stasiuk, 2011).

The insecticidal potential of CNSL on insects of agricultural importance has not been exhaustively reported (Ferreira de Carvalho et al., 2019) thus reinforcing the relevance of the present study. Furthermore, the insecticidal potential of CNSL has not been utilized fully (Kala et al., 2019) in spite the fact that it is safe towards non-target organism (Mukhopadhyay et al., 2010). However, some studies have been carried out on the anti-insect activity of CNSL against mosquitoes (Vani et al., 2018; de Carvalho et al., 2019) and crawling insects (Buxton et al., 2017; Buxton et al., 2018). Hence our study on the insecticidal activity of CNSL against *Bactrocera dorsalis* is an area that has limited investigation thus making our study as a gap filling endeavour.

1.2 Objectives

Therefore, this thesis is focused on the effects of CNSL against the Asian papaya fruit fly *Bactrocera dorsalis.* This study had the following specific objectives:

1. To investigate the toxicity of dichloromethane and hexane CNSL extracts on adult male *B. dorsalis* through direct contact action.
2. To determine the insecticidal action of dichloromethane and hexane CNSL extracts on adult male *B. dorsalis* through topical application.
3. To investigate the repellent action of dichloromethane and hexane extracts of CNSL on adult male *B. dorsalis*.
4. To investigate the effectiveness of CNSL treated baits with response of colours and methyl eugenol (ME) against *B. dorsalis* in field testing.

CHAPTER 2

LITERATURE REVIEW

2.1 Pests of papaya

There are 134 species of arthropods that attack papaya. Most of the species are insects in the following orders and families Hemiptera 9 species in 2 families attacking fruits and leaves; Homoptera 65 species in 11 families attacking fruits, foliage, trunk and some are vectors; Thysanoptera 4 species in 1 family attacking foliage, leaves fruits, and are vectors; Coleoptera 7 species in 4 families some are foliage feeders, root feeders, and others attack the trunk; Diptera 26 species in 1 family with some attacking the seeds, and others the fruits which include *Bactrocera* spp; Lepidoptera 11 species in 4 families some members of this order are foliage feeders, fruit feeders and some attack the trunk; Acari 12 species some of these pests, attack the fruits, trunk and others are disease vectors.. All the dipteran species are fruit flies in the family Tephritidae. (Peña et al., 2002)

2.2 *Bactrocera* spp.

The genus *Bactrocera* Macquart (Diptera: Tephritidae) is recognized worldwide for its destructive impact on agriculture. Apart from causing billions of dollars in direct losses to a wide variety of crops it also limits the development of agriculture in many countries due to reduction in farm size, income. It also leads to overuse of pesticides (Danjuma et al., 2014). Numerous fruit fly species constitute enormous threats to fruit and vegetable production throughout the world, causing both quantitative and qualitative losses. Furthermore, due to their susceptibility of many fruits to invasive tephritid species, many fruit-producing countries have imposed quarantine restrictions on the import of products from countries infested with particular fruit fly species, and/or require that fruits and vegetables undergo quarantine treatment before their importation is allowed (Vargas et al., 2015). The damage which results from their infestation, if uncontrolled, may result in a total crop loss (Yong et al., 2010).

The *Bactrocera* spp. are known to be largely endemic to Asia and the Pacific. They are among the serious pest species, and many of them are indigenous to Thailand and Peninsular Malaysia and are members of the *Bactrocera dorsalis* complex which, include *B. dorsalis* Hendel, *Bactrocera carambolae* Drew & Hancock, *Bactrocera papayae* Drew & Hancock, and the cucurbit feeders *Bactrocera cucurbitae* (Coquillet) and *B. tau* (Walker) (Ullah et al., 2015).

Fruit flies cause direct damage through the puncture by the female for oviposition and the subsequent larval development that takes place inside the affected fruits. The larvae destroy the internal tissues of the fruits resulting in premature ripening before becoming rotten and falling to the ground. Because of this behaviour, these pests cause losses of 40% to 80% if no remedial action is taken (Kibira et al., 2010). The presence of these pest species limits access to international markets due to quarantine restrictions imposed by importing countries (Lanzavecchia et al., 2014).

2.2.1 Economic importance of *Bactrocera* spp in papaya production

Ripe papaya is consumed fresh as a dessert fruit while green (unripe) papaya is added into fresh salads. Papain is an important bioactive substance recovered from the latex of the green fruit. Fairly easy to grow, the plant is commonly planted in home gardens using seeds. Parts of the plant that have use include leaves, fruit, seed, latex, and root. Some of these parts are known to be analgesic, amoebicidic, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifugic (Anibijuwon & Udeze, 2009).

Two potent biochemically active compounds of the papaya are chymopapain and papain, which are known to aid digestion. Papain is active over a wide pH range and is employed in treating arthritis, dyspepsia, and other digestive disorders, and prepared in liquid form for reducing enlarged tonsils. Dietary papaya intake reduces human urine acidity. Chymopapain has been used, of lately for intradiscal injection in patients with documented herniated lumbar inter-vertebral discs. In addition, papaya leaves are smoked for asthma relief and it's used for nervous pains and the treatment of elephantiasis growths. Young leaves contain carpain an active bitter alkaloid which has a depressing action on heart (Boshra & Tajul, 2013).

The fruits are low in calories and rich in natural vitamins and minerals. Papaya places the highest among fruits for vitamin C, vitamin A, riboflavin, folate, calcium, thiamine, iron, niacin, potassium and fibre (Boshra & Tajul, 2013). It is a good source of serotonin (0.99 mg/100 mg), which has been associated with enabling the gut to mediate reflex activity and also decreasing the risk of thrombosis (Santiago-Silva et al., 2011).

Many biologically active phytochemicals from different parts of papaya tree (latex, seed, leaf, root, stem, bark and fruit) and studied their potency. Hence, papaya fruits are used as topical ulcer dressing in some hospitals including the Spanish town hospital, Kingston public hospital and university hospital in the West Indies, Jamara, and the dressing preparation from papaya promotes healing and reduces odour in chronic skin ulcer. Furthermore, some hospitals use it as burn dressing which is tolerable by children and is economical and widely available (Krishna et al., 2008).

Plate 2.1 Raw cashew nuts

2.2.2 *Bactrocera dorsalis*

The *Bactrocera dorsalis* species is a group of true fruit flies (Diptera: Tephritidae) that consists of approximately 100 morphologically similar taxa (Drew & Romig, 2013). The Oriental fruit fly, *B. dorsalis* (Hendel), and closely associated species in this group, namely the Asian Papaya fruit fly, Drew & Hancock, the Philippine fruit fly, *Bactrocera philippinensis* Drew & Hancock have been classified as the same species and hence Schutze et al. (2015) synonymized this group as one biological species referred to *B. dorsalis* complex.

It is largely recognized that the native region range of *B. dorsalis* is Southeast Asia (Choudhary et al., 2016) or southern East China (Wan et al., 2011) from where it spread to more countries. Over the last century, *B. dorsalis* migrated from southern East China or Southeast Asia to other places of Asia, Africa and the Americas, a pathway that has been well supported by previous research (Wan et al., 2012; Aketarawong et al., 2014). In confirmation of the generally accepted view that *B.*

dorsalis originated from South East Asia. Aketarawong et al. (2014) research using microsatellite technology confirmed that *B. dorsalis* possibly originated from southern East China and migrated in a westward-oriented invasive route from its native range, with acolonization process associated with relatively stable population demographics of adventive populations, especially in an unfragmented habitat, rich in intensive cultivation such as in Southeast Asia. Similarly, Wan et al. (2012) reconstructed the invasion history of *B. dorsalis* in the Asia Pacific region using molecular markers from mitochondrial genes. They projected two main invasion routes: from southern East China to central China, and another from southern East China to other counties in south- eastern Asia. Shi et al. (2012) combining both mitochondrial *COI* sequences and nuclear DNA markers, used a large area sampling of *B. dorsalis* including other Southeast Asian countries and central China concluded that the tropical regions of Southeast Asia and the southern coast of China were the native range of *B. dorsalis*, which then expanded northward up to central China and eastward to Yunnan. Li et al. (2018) reported that by 2017, *B. dorsalis* had been detected in four continents (Asia, Africa, North America and South America) and Oceania, including 75 countries.

2.2.3 Biology of *Bactrocera dorsalis*

Amur et al. (2017) studied biology and morphometric of different life stages of the oriental fruit fly in Pakistan, reported that the eggs of *Bactrocera dorsalis* were white, shiny, rice-shaped, slightly curved into elongate tapering at the anterior and posterior end which varied from 0.5mm-0.6mm with mean of 0.54±0.11mm and width 0.1-0.3mm with mean 0.19±0.08mm.

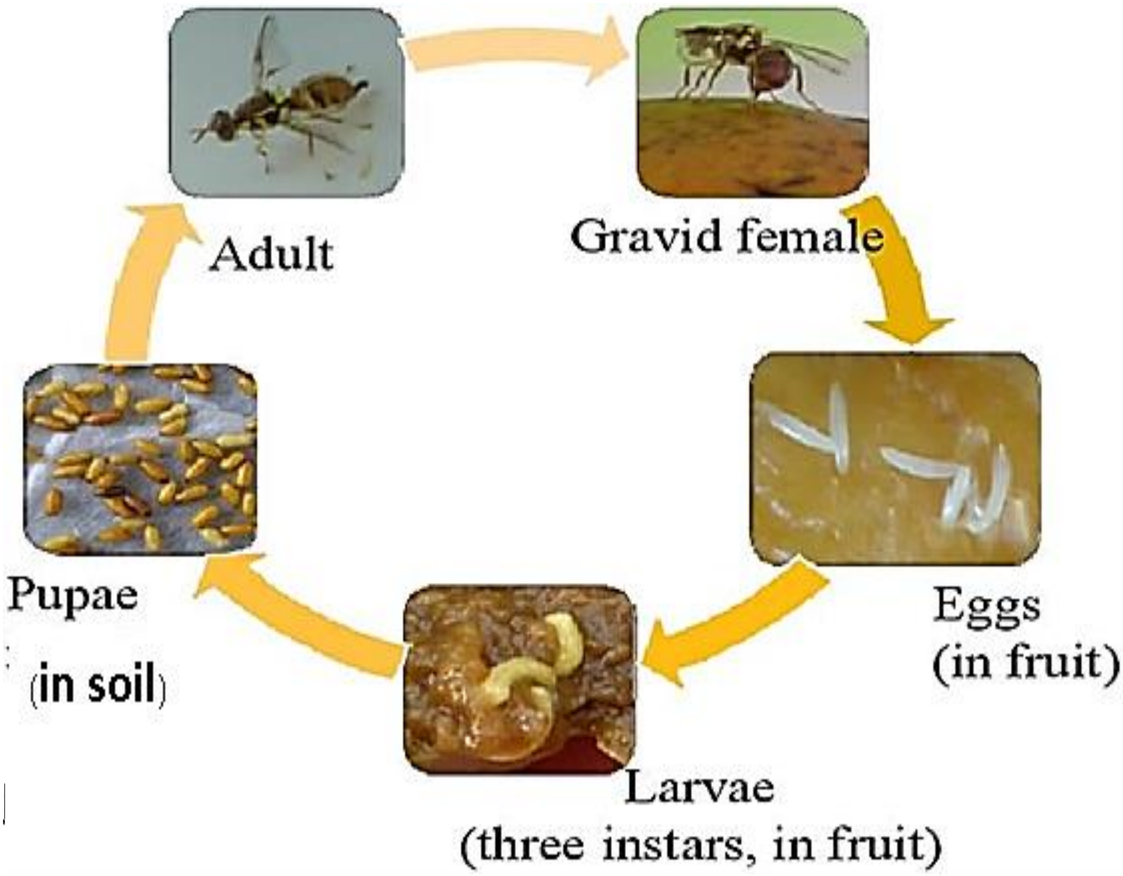

Plate 2.2 Life cycle of *Bactrocera dorsalis*

The egg-laying and hatching period are 1-2 days with an average of 1.61 ± 0.51 days. The larval stage passes through three instar phases (1st, 2^{nd,} and 3rd stages). The 1st instar a transparent and creamy/white in colour with length 1-2mm (2.6±0.75mm) and width 0.2-0.4mm (0.27±0.82mm). The 2nd instar's length was 5-6mm (0.55±5.88), and width 2-3 (2.34±0.78mm). The fully grown 3rd instar larvae had some visible characters; the head was pointed anteriorly with well-developed mandibles, hooks, spiracles on both anterior and posterior sides of the body and black mole on the anterior and caudal side. With a length of 78mm (7.68±0.72 mm) and width of 3-4mm (3.58±0.25mm). The authors observed that the development period was 8-10 days with mean values of 9.97±2.25 days.

The size of pre-pupae was 2-3mm an average of 2.93±0.49m and width 2-4mm with an average of 3.89±0.20mm. The developmental period was 1-2 days. Pupae emerged in a segmented and cylindrical dark brown hard capsule. They measured 4-5mm in length with average (4.47±0.64mm) and width were 2-3mm with average (2.69±0.16 mm). Duration of pupae stage varied from 8 to 9 days with a mean of 8.52±0.88. Adult flies emerged from pupae within 8.9 days. Adult flies can live for 30-45 days (Amur et al., 2017)

2.2.4 Control methods for papaya fruit flies

The papaya fruits are eaten raw shortly after being harvested from the field. The sole control strategy totally relying on insecticides becomes complicated against papaya fruit flies (Dhillon et al., 2005). This is due to the fact that some insecticides take a long time to degrade. Several control methods have been reported for papaya fruit flies that are integrated to reduce the damage done by the pest and prevent the likelihood of residual toxicity that may arise from insecticide usage.

2.2.4(a) Cultural control

Cultural methods of control generally rely upon orchard sanitation and crop hygiene which is focused at disrupting the normal life cycle of the target pests. Cultural control comprises the collection and destruction of all the infested fruits either they are present on the trees or fallen on the ground. Destruction of fruits can be done by crushing them in a grinder followed by burying them under the soil surface at least >50 cm depth (Devi & Komala, 2020).

2.2.4(b) Chemical control

Chemical or the insecticidal methods of control of fruit flies fall under three main categories: spray the adults with suitable insecticides, trapping of the adult flies through a chemical attractant and bait spray, and insecticide mixed with bait (Kotikal & Math, 2017; Vargas et al., 2015).

Because much of the damage by fruit flies occurs when females lay their eggs and larvae develop within fruits. Followed by pupation usually occurring in the soil the main control methods for fruit flies involve treatment of adults with chemical insecticides (Hsu, 2015). The history of fruit fly control started with full cover sprays using inorganic insecticides (e.g., lead arsenate) in the early 1900s and continued through the century with a transition to synthetic insecticides, such as chlorinated hydrocarbons, organophosphates, and synthetic pyrethroids (Daane & Johnson, 2010; Vargas et al., 2015).

Organophosphates kill adult flies through contact and baits (Dominiak & Ekman, 2013), can penetrate the fruits and kill eggs and young larvae. Consequently, they have an advantage in keeping fruit infestation to a minimum. They have been applied as cover sprays usually as the only control method against fruit flies. (Rahman & Broughton, 2016).

In China, chemical insecticides are still used as the primary measure to control populations of Oriental fruit fly (Liu et al., 2019). However, insecticides such as malathion, trichlorfon, endosulfan, abamectin, spinosad, β-cypermethrin, and pyrethroid are moderately effective against this fruit fly in most orchard fields (Jin et al., 2011). Dichlorvos (at 600 g a.i.) is reportedly effective for controlling this fly. Trichlorfon, at a concentration of 1:1 000 in combination with 3% brown sugar, reduced the fruit fly population by approximately 90% more than the untreated control furthermore, the control effect reached 71.9% using chlorpyrifos in a recent field test (Liu et al., 2019).

2.2.4(c) Trapping

The major challenge in fruit fly control is that the process of egg-laying is done in the fruits and tender vegetables, and maggots develop inside, which are beyond the reach of insecticide sprays. Therefore, the management tactics must be directed mainly in the pre-oviposition stage when the female flies require plenty of water to drink and proteins for egg maturation. As a result, this behaviour makes them be easily attracted to solutions or syrup. Female tephritids require a protein meal for ovarian development and egg production. This habit of the flies has been taken advantage of through the use of traps with poisoned food (Kotikal & Math, 2017).

Traps, attractive lures, and mass trapping techniques are common strategies for managing fruit flies Vargas et al. (2009). (Epsky et al., 2011) reported that McPhail baited traps successfully trapped fruit flies in a field study carried out in Florida. The trapping technique operates on the principle of using a lure to attract a pest organism to a point where it can be killed (El-Sayed et al., 2009). The killing device is generally an insecticide mixed with, or placed adjacent to, the lure, but alternatives include liquid traps where the pest enters and drowns or sticky traps which hold the insect until it dies. The lure itself can be a semiochemical (including pheromones, kairomones, and food- based volatiles), nonvolatile food attractants, colour attractants, host mimics, or a combination of these (Clarke et al., 2011).

In a study by Danjuma et al. (2014) on the seasonality of *B. papayae* on guava in peninsular Thailand, an average of over ten thousand insects was trapped from six traps in urban areas and similarly trapped a combined average of over 16,000 insects from 24 traps over a period of 53 weeks, using a modified Steiner traps with cotton wool soaked with a mixture of methyl eugenol used as an attractant and a pyrethroid.

2.2.4(d) Baits

The management of fruit flies is primarily done through the spraying of chemical insecticides such as organophosphates. This method of controlling fruit flies has resulted in the development of insecticide resistance, pest resurgence, environmental pollution and food contamination through increased residual level thus becoming a health hazard (Dias et al., 2018). Another drawback to the use of chemicals is that 3rd instar larvae leave rotten fruits and drop to pupate in the ground soil. Therefore, eggs and larvae in fruits and pupae in soil are well protected from insecticide surface application (Heve et al., 2017). Hence the use of baits is now one of the most important preventative methods (Epssky et. al., 2014). Furthermore, attract and kill tools based on the use of food attractants (protein baits) or semiochemicals coupled with an insecticide have been proposed as a novel control tool in tephritid (Canale et al., 2013). Baits are effective in that fruit flies require protein for reproductive development and feed upon protein in the field. Hence, in natural systems, fruit fly adults can feed on a wide variety of foods such as floral nectar, insect honeydew, leaf and fruit exudates, and other organic matter sources such as yeast, bacteria and bird faeces (Daane & Johnson, 2010; Dong et al., 2014).

This behaviour is utilized to control fruit flies by mixing an insecticide with food attractants, and the technique is commonly referred as insecticide bait sprays. The method aims to provide effective management in that the food source is poisoned so that the flies are killed when they come in contact or feed on the bait (Kotikal & Math, 2017). For example, Singh et al. (2013) reported that continuous trapping of fruit flies (*Bactrocera* spp. males) in 20-25 year old mango orchard during 2006-08 season by using parapheromone-insecticide lure (0.1% methyl eugenol + 0.05% malathion) traps at 10 traps/ha, fruit fly catches declined significantly (>80%) in 2007 as compared to the previous year.

Similarly, Gonçalves et al. (2012) reported that the bait spray was as effective as dimethoate which it was compared with, and that it was found to have a mild and favourable toxicological and ecological profile. Thus, making it a good alternative for the control of the pest in Continental Mediterranean climate such as in Terra Quente.

2.2.4(e) Male sterile technique

The Sterile Insect Technique (SIT) it is defined as a method of pest control using area-wide flooded release of sterile insects to reduce reproduction in a field population of the same species. SIT represents therefore a type of birth control in which wild female insects do not reproduce when they are inseminated by released sterilized males (Gnilane et al., 2022). It relies on the rearing of the target insect in large numbers in specialized production centres. One of the sexes was, facing sterilization with ionizing radiation and then release over the target area (Maru & Sao, 2018).

In Spain a reduction of more than 90% of a target wild population was achieved based on the sterile insect technique (SIT), which was implemented from 2003 to 2006. As a result, there has been a reduction of more than 90% in the use of insecticides by aerial means to control *C. capitata*, as well as a growth trend in exports of citrus and fresh fruits from the Valencian Community in recent years (Pla et al., 2021). Hence in some regions of the world methods like sterile insect technique (SIT) has been used for successful population suppression/eradication of fruit flies (Agarwal, 2019).

2.2.4(f) Biological control

In recent years, due to the undesirable effects of chemical pest control, the use of insecticides such as organophosphates and pyrethroids have been restricted (Grdiša & Gršić, 2013). As a result of this decision, tephritid fruit fly management with emphasis on *Bactrocera* spp in many economically important crops have been seriously impacted through phenomenal losses due to pest damage. Consequently, in order to reduce the volume of losses that may be incurred following this decision, the management of tephritid fruit flies require a holistic approach of which biological control is one of the essential components (Samira et al., 2016). Hence combining biological control with SIT as a control strategy against tephritid fruit flies is being strongly being promoted (Gurr & Kvedaras, 2010). However, regardless of the fact that a lot of research has been done in this area, biological control accounts for less than 1% of the global sum of US$30 billion of all control methods used in agriculture (Paranhos et al., 2019). However, research conducted in Hawaii where a large population of parasitoids was released resulted in the reduction of population to up to 95%. Among the fruit fly parasitoids, *Fopius arisanus* (Hymenoptera: Braconidae) has a competitive advantage because it attacks eggs laid under the fruit skin, which are easier to reach than larvae deep in the pulp; therefore, theoretically, it could out-perform other species (Coelho, 2017).

The first classical biological control programs against fruit flies were conducted in Hawaii against *Bactrocera* spp. It involved the introduction of parasitoid and predator species not only for the control of this pest but also for using them against other pestiferous tephritid species (Garcia et. al., 2020). Thus the introduced parasitoid species, *Fopius ceratitivorus* has become an established parasitoids in the Hawaiian Islands (Kroder & Messing, 2010).

2.3 Impact of synthetic insecticides

Synthetic insecticides have significantly benefited farmers over the years; however, the major disadvantage with their application have been the issue of environmental damage. They are known to contamination of water, air, and soil resources, toxic to non-target organisms and humans due to the presence of pesticide residues in food, and the development of pest resistance (Kumar, 2012; Fountain & Wratten, 2013).

Furthermore, synthetic pesticides can reach the soil through direct applications, such as seed treatments, indirectly by applying to the aerial parts of plants, the falling of treated foliage or fruits, and the movement of contaminated water on the surface and within the soil profile (Chaplain et al., 2011; Cycoń et al., 2017).

It was reported by Meite et al. (2018), Salazar-Ledesma et al. (2018), and Shaheen et al. (2017); that once there is a build-up in the soil, these chemicals are transported by leaching and surface runoff, and they can undergo chemical processes such as hydrolysis, photolysis, and chemical degradation, as well as they can interact with the living fraction of the soil and be biodegraded (Chaplain et al., 2011). Consequently, synthetic pesticides and/or their metabolites can reach hydric resources, become bioaccumulated through the food chain, be completely mineralized, or persist for long periods in the soil (Chaplain et al., 2011).

Cycon et al. (2017) reported that the continuous use of agrochemicals on a large scale leads to their buildup in the soil which has a negative impact on the soil microorganism. As a result, microorganisms respond in a variety of ways to the different types of pesticides applied and may exhibit increased or inhibited growth and metabolism.

Many studies have shown that pesticides cause qualitative and quantitative changes in the soil microbiota (Komorowicz et al., 2010; Hartmann et al., 2015), leading to alteration of nitrogen cycling (Damin & Triveli, 2011), changes in soil enzymatic activity, and disruption of the symbiosis between mycorrhizae and root nodules in legumes. These factors lead to alterations in soil fertility and, consequently, plant growth (Malik et al., 2017).

2.4 Botanical insecticides as an alternative control

Terrestrial plants have been known to be a rich source of novel compounds which act as defensive agents against insects and other herbivores and plant pathogens. Hence they have demonstrated bioactivity against insects which can be broadly categorized as behavioural (repellence, feeding deterrence, oviposition deterrence) or physiological (acute toxicity, developmental disruption, growth inhibition) Isman, 2017. Many plant-based pesticides have been utilized as insect repellents, antifeedants, insecticides, and insect growth inhibitors. In addition, some of these botanical bioactive ingredients are applied as nematicides, fungicides, bactericides, and virucides The majority of plant-derived compounds are used to control insect pests (Mkenda et al., 2015; Waziri, 2015; Ngegba et al., 2022) Botanical pesticides could be useful as alternative tools for integrated pest management since they have positive impacts on environmental preservation, low toxicity to mammals, and low risk of developing resistance in target pests (Essiedu et al., 2020). However, despite the much talked about benefits of botanical insecticides, these sizeable sources of botanicals have been underutilized and neglected as pesticidal agents to manage a plethora of destructive pests and diseases. Thus, the application of botanicals as pesticidal agents and their effectiveness as alternative pest management in sustainable agricultural and in related fields have been utilized to the maximum (Ngegba et al., 2022).

2.5 *Anacardium occidentale L.*

Cashew (*Anacardium occidentale* L.) belongs to the family Anacardiaceae. It is considered native to the northern part of South America (Brazil), and it is now found in many tropical areas. Cashew is an evergreen perennial tree, and growth is not continuous throughout its life. Instead, growth occurs as intermittent short-lasting flushes of shoots from apical or lateral buds of resting stems before returning to a dormant state. Periods of extended dormancy are generally short in young plants. However, it usually lasts several months between flushing episodes in mature trees (Malhotra et al., 2017).

Cashew and its products are curative to many human health problems and offer a variety of advantages to the human body (Tola & Mazengia, 2019). Cashew nut helps to lower the cholesterol level in the blood, control diabetes and coronary heart disease risk (Desai et al., 2017; Ros, 2010). They are rich in magnesium which is vital for healthy bone development and prevention of high blood pressure (Dendena & Corsi, 2014). Furthermore, the plant also has antibacterial and anthelmintic properties (Tola & Mazengia, 2019). Griffin & Dean (2017) reported that despite the lower levels of unsaturated fatty acids in the cashews compared to other tree nuts, consumption of cashews could reduce the risk of cardiovascular disease. Cashew apple extract, cashew nut shell crude extract, and bark gum extract are used as insecticides (Buxton et al., 2017) and antifungal products (Morais et al., 2017).

2.5.1 Cashew nut shell liquid (CNSL)

Cashew nut shell liquid (CNSL) is an economically important by-product obtained from the cashew nut *Anacardium occidentale* L. (Anacardiaceae). Cashew nut shell liquid is a substance contained between the kernel`s inner and outer shells in a honeycomb matrix (Stasiuk, 2011). It is a caustic, viscous dark pericarp liquid (Buxton et al., 2018). An oily or balsams are the substance of relatively high-volume mass with a bitter taste, caustic property and, when heated, gives off pungent and choking fumes (Malhotra et al., 2017). Stasiuk (2011) reported that a typical solvent-extracted CNSL contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%) and traces of 2- methyl cardol. Cashew nut shell liquid has found various applications in industrial technology. Due to its phenolic nature, this product can be used directly as an excellent preservative for timber and certain crude fiber textiles against insect and fungus attacks (Malhotra et al., 2017). Furthermore, natural CNSL has been found to be versatile with a wide range of uses, including as a raw material in the polymer industry, in the development of drugs (antioxidants) and in pest control. It is also known to be toxic and corrosive to the skin (Idah et al., 2014).

2.5.1(a) Bioactive compounds in CNSL

a. Anacardic Acid (AA)

Anacardic acid (AA) is a bioactive phytochemical found in the nutshell of *A. occidentale*. Chemically, it is a mixture of several closely related organic compounds, each consisting of salicylic acid substituted with an alkyl chain. Traditional medicine practitioners in India use nutshell oil as a medicinal remedy for malaria and other ailments. Current research and emerging evidence suggest that AA could be a potential target molecule with bactericidal, fungicidal, insecticidal, anti-termite and molluscicidal properties and as a therapeutic agent in the treatment of the most serious pathophysiological disorders like cancer, inflammation and obesity (Hemshekhar et al., 2012).

b. Cardol

Ethanol crude extract of *A. occidentale* L. displayed significant anti-plasmodial activity. When the main isolated compounds were evaluated on *Plasmodium falciparum* D6 strain, the results showed cardol to possess a good antimalarial activity (Gimenez et al., 2019).

c. Cardanol

Cardanol is one of the main constituents of natural Cashew Nut Shell Liquid (CNSL), obtained by solvent extraction and assayed for antioxidant, larvicidal and antiacetylcholinesterase activity. Its antioxidant activity showed it to be very active and has been found to possess good larvicidal activity against *Aedes aegypti*. It is a promising agent in the control of *Ae. aegypti*, the main dengue vector in Brazil (Oliveira et al., 2011).

Buxton et al. (2018) reported that exposure of the red rust flour beetle *Tribolium castaneum* to cardanol produced 80% mortality of adults in the insecticidal bioassay. In the progeny growth and development inhibition bioassay, 46.7% of larvae were killed, and only 20.7% of adults emerged without deformities after the cardanol treatment.

d. 2-Methyl cardol

A study by Alvarenga et al. (2016) reported that ethanol extract of *A. occidentale* confirmed 2-methylcardol was active against *Schistosoma mansoni* adult worms in vitro. Scanning electron microscopy of the tegument of male worms showed severe damage as well as peeling and reduction in the number of spine tubercles. In addition, worms had lysed interstitial tissue, degenerated mitochondria, and drastically altered tegument.

2.6 Extraction method of plant extract

Plant extracts are obtained using a variety of solvents and techniques for extracting different compounds produced by the plant (Tiwari et al., 2011). Properties of a suitable solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to be complex or dissociate (Pandey & Gupta, 2020).

The choice of solvent is influenced by what is intended with the extract. Since the product will contain traces of residual solvent, the solvent should be non-toxic. It should not interfere with the bioassay since, during extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Das et al., 2010). What should be borne in mind in choosing a solvent for extraction is polar solvents will extract polar molecules and non-polar solvents will extract non-polar molecules (Shaalan et al., 2005; Ravi et al., 2018). Hence the yield of chemical extraction depends on the type of solvents with varying polarities, pH, extraction time and temperature as well as on the chemical compositions of the sample (López et al., 2011). Ghosh et al. (2012) reported that the extraction of active biochemical from plants depends upon the polarity of the solvents used. Thus, polar solvent will extract polar molecules and non- polar solvents extract non-polar molecules. This was observed when eleven solvent ranging from hexane, petroleum ether the most nonpolar (polarity index of 0.1 that mainly extracts essential oil) to water the most polar (polarity index of 10.2) which extracted compounds with higher molecular weights such as proteins and glycans. Chloroform or ethyl acetate were found to be moderately polar (polarity index of 4.1) steroids, alkaloids, were extracted with these solvents. Most studies use solvents with minimum polarity such as hexane or petroleum ether or those with maximum polarity such as aqueous steam distillation. For extraction of lipophilic compounds, dichloromethane or a mixture of dichloromethane and methanol in the ratio of 1:1 are used (Sasidharan et al., 2011).

Botanical extracts that exhibit bioactivity against pests can be grouped into five major chemical categories: nitrogen compounds, terpenoids, phenolics, proteinase inhibitors, and growth regulators (Showler, 2017). Cashew nut shell liquid is lipophilic and the compounds extracted are phenols.

2.6.1 Extraction procedures

Plant tissue homogenization: Plant tissue homogenization in the solvent has been a widely adopted procedure used by researchers in plant extraction. It has the advantage of improving the kinetics of analytic extraction and also increasing the contact of the sample surface with the solvent system (Sasidharan et al., 2011). The method involves dried or wet; fresh plant parts being ground in a blender to fine particles before putting in a certain quantity of solvent and shaken vigorously for 5-10 minutes or left for 24 h after which the extract is filtered. The filtrate may then be dried under reduced pressure and redissolved in the solvent to determine the concentration. However, some researchers may choose to centrifuge the filtrate to clarify the extract (Das et al., 2010). A disadvantage that could be associated with this method is the dust from ground dry plant parts that could cause allergic reactions, irritation to the eyes or respiratory problems to the researcher.

Serial exhaustive extraction: It is another common procedure of extraction which involves successive extraction with solvents in ascending order of polarity from a non-polar to a more polar solvent to ensure that a wide polarity range of compounds is extracted (Jaradat et al., 2015). It has the advantage of being able to ensure that a wide polarity range of compounds could be extracted (Banu & Cathrine, 2015). A major weakness of this method is it cannot be used for