ASSESSING THE POTENTIAL OF THE USE OF LOCALLY-AVAILABLE SPENT BREWERY GRAIN IN THE MANAGEMENT OF FRUIT FLIES IN SOUTHERN GHANA

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DECLARATION

I, Attafuah, Michael Agyeman hereby declare that this submission is my own work towards the award of a Master of Philosophy Degree in Entomology at the African Regional Postgraduate Proramme in Insect Science (ARPPIS), University of Ghana, and that to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

The efficacy of Spent Brewery Grain of Pito (SBGP) and Brukutu (SBGB), used as sources of protein baits, were compared with two standard products - SUCCESS Appat (GF-120) and Great Fruit Fly Bait (GFFB)) as attractants in the field for the management of fruit flies in four mango farms in the Coastal Savanna ecological zone (Christian Farms, Adom Farms, Prosper and Boko Farms). Five plots (SBGP, SBGB, GF-120, GFFB and Control) were set up in each farm, with a total of 20 plots used for the study. Preparation of the four food bait treatments were delivered as spot sprays of approximately 50ml onto one square meter of foliage per tree on weekly bases, with Control plots receiving no treatment. After eight (8) weeks of treatment applications, thirty (30) mango fruits from each plot were harvested, weighed and incubated on sterilized sand to determine their levels of infestation. A total of six hundred mango fruits, weighing 400.66 kg were used for the incubation. The highest infestation level of 1.119 puparia per kg of fruits was recorded in the control plot, followed by 0.492 (GF-120), 0.458 (SBGP), 0.272 (SBGB) and 0.113 (GFFB). These results translate into fruit protection levels of 59.07 % (SBGP), 75.69 % (SBGB), 56.03 % (GF-120) and 89.90 % (GFFB), compared to the control plot. This implies that continuous use of local Spent Brewery Grains of Pito and Brukutu when integrated with other management practices such as orchard sanitation can help control fruit fly attack. Population dynamics of fruit flies were also monitored on weekly basis in the four different fields. A total of 155,180 insect species were collected during the study period, of which 152,575 (98.32%) were fruit flies and 2,605 (1.68%) as non-target organisms.

DEDICATION

I dedicate this work to Prophet Akoa Isaac, the founder and leader of the Church of Prosperity, his wife and his family.

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LIST OF ABBREVIATIONS

ACP	African, Caribbean and Pacific Countries
ARPPIS	African Regional Postgraduate Programme in Insect Science
AWCP	Area Wide Control Program
BAT	Bait Application Technique
CUE	Cuelure
DABCS	Department of Animal Biology and Conservation Science
DDVP	Dimethyl 2, 2 – DichloroVinyl Phosphate
EUSMG	European Union Strategic Marketing Guide
FFEPO	Fruit Fly Eradication Project Office
FTD	Fly per Trap per Day
GEPC	Ghana Export Protection Counsel
GFFB	Great Fruit Fly Bait
GNA	Ghana News Agency
IAEA	International Atomic Energy Agency
ICIPE	International Centre of Insect Physiology and Ecology
ITFC	Integrated Tamale Fruit Company
IPM	Integrated Pest Management
MAT	Male Annihilation Technique
ME	Methyl eugenol
MOFA	Ministry of Food and Agriculture
Mt	Million Tonnes
MWB	Mineral Water Bottle

NRI	Natural Resource Institute
OMRI	Organic Materials Review Institute
RCBD	Randomized Complete Block Design
RH	Relative Humidity
PPRSD	Plant Protection and Regulatory Services Directorate
SBGB	Spent Brewery Grain of Brukutu
SBGP	Spent Brewery Grain of Pito
SIT	Sterile Insect Technique
TA	Terpinyl acetate
TML	Trimedlure
USAID	United State Agency for International Development
US $	United State Dollar
WAFFI	West Africa Fruit fly Initiative

CHAPTER ONE

1.0 GENERAL INTRODUCTION

The fastest growing sector of the agriculture industry in sub-Saharan Africa is the fruit and vegetable crop production. The growth of this sector may be as a result of changing dietary patterns leading to increased consumption of fruits and vegetables (Lux *et al.*, 2003a). The sector has the potential to provide nutrients to supplement daily dietary requirements, income, provide employment and also earn foreign exchange through export (Ekesi and Billah, 2006). In the African, Caribbean and Pacific (ACP) countries, up to 45 million people depend on vegetables and fruits for export to Europe alone (Braun, 2002) as estimated by the Natural Resource Institute (NRI). A wide range of fruits including water melon, avocado, citrus, mango, pineapple, guava and apple, are among the most common fruits grown for export to the European Union, Middle East and for domestic urban markets (Ekesi and Billah, 2006). According to the FAOSTAT (2013), fruits are exported as either fresh or processed to many countries in Europe, Middle East and North America. Of all the tropical fruits produced worldwide, mango, a crop which originates from South-East Asia, constitutes about 50% (Stefan *et al*., 2003). Mango fruits are eaten raw and serve as an excellent source of dietary fiber, provitamin A and vitamin C (Evans, 2008). According to the Food and Agricultural Organization of United Nations (FAO) (2010), India is the world's largest producer of mango with an estimated production of 15 million metric tons, followed by China with 4.3 million metric tons. In 2015, the World export of mango was estimated to increase to 1.4%, totaling 844,246 tons. Mango production and export in the West Africa sub region to the European market was estimated to have increased from 15,000 to over 22,000 tons, indicating a rise of 45% in 2012 (ECOWASTEN Newsletter, 2012).

The total land area under production of mango in Ghana was estimated to be 4,166.7 ha in 2006 and 6,360 ha in 2008 (Adongo, 2006; Stonehouse *et al*., 2008; MOFA, 2010). According to ECOWAS-TEN Newsletter (2012), production of mango in Ghana increased from 144 tons in

2011 to about 585 tons in 2012. This significant export volume of mango from Ghana to the European and Asian markets far exceeds the demand (Avah *et al.*, 2008). This shows clearly that there is a potential to increase production of mango in Ghana.

Fruit flies are spread from one region to another through international movement of infested fruits, and this has made the African continent a home and point of introduction of several fruit flies species (Ekesi and Mohamed, 2010). Alien fruit fly species invasions such as, *Bactrocera zonata* in Egypt (in 1997); *B. dorsalis* in Kenya (2003), in Ghana in 2005 (Billah *et al*., 2006) and *B. latifrons* in Tanzania (in 2006) are the cause of numerous horticultural production problems. These are suspected to have arrived on the African continent through trading in fruits and vegetables. *Bactrocera dorsalis* attacks a wide range of fruits and vegetables (Lux *et al*., 2003b; Drew et al., 2005; Billah *et al*., 2006; Ekesi and Billah, 2006), and has been described by the African Union as a "devastating quarantine pest" (French, 2005). However, mangoes exported to some European countries are intercepted, confiscated, and destroyed because of the presence of insects considered as quarantine pests, thus leading to huge economic losses to the horticultural sector and the exporters of most of the exporting countries including Ghana (ACP-EU, Newsletter 2013). Over 90 interceptions of mango were made from the sub-region, Cote D'lvoire, was the highest which led with 34 interceptions, followed by 28 interceptions from Ghana. The cost of this was valued at US$3.67 million at a rate of US$39,348 per interception in 2012 (ECOWASTEN Newsletter, 2012). Importing countries like the United States and the European Union threaten to impose a ban on Ghana mango since the beginning of 2012 due to the presence of insect pest. In Ghana, the horticulture industry lost over US$10 million in 2013 when the US finally banned the export of fruit from Ghana (GEPC, 2014).

Among all the insect species in the tropics, fruit flies are the major pests of several cultivated fruits and vegetables, with the growing international trade further increasing the significance of

fruit flies (Allwood, 1997; Lux *et al*., 2003a, b; Ekesi and Billah, 2007; Yeboah, 2012). To improve the productivity and quality of fruits and vegetables which could ensure food security, employment and trade opportunities, these fruit fly species should be successfully controlled (Fruit fly info, 2010). Early detection is the best means to achieve speedy and cost-effective eradication of fruit flies, and traps are among the most used tools for fruit fly monitoring. Attractants such as Cue lure, protein baits and Methyl eugenol, are used together with traps for determining the presence of fruit flies in a region or country. Orchard sanitation, the use methyl eugenol, insecticide sprays and poisoned protein baits are some of the most effective control measures when used in an integrated pest management programme (Bugs for bugs, 2013). Considering insecticide spray and protein bait control strategies used, the bait system is more acceptable for an integral control due to the minimal pesticides use and its target-specificity (Dekker and Messing, 1999). Adult fruit flies require protein for development and growth, and therefore protein baits hold a greater potential for fruit fly control than insecticide sprays which are non-specific. According to the Secretariat of the Pacific Community (2002), protein baits are food attractants and their effectiveness relies on the fact that immature males and females require protein for reproductive development. Fruit flies detect the protein source over short distances (10-15 m), but some commercial baits are combined with other volatile compounds to make them attractive over greater distances (AFFP, 2014). Their use limit the quantity of insecticides used, leaving lower residues in the environment and on crops, and causing little or no harmful effect to parasitoids and pollinators (Galun *et al*., 1983), and thus pose little threat to non-target organisms. Furthermore, small amounts of insecticides incorporated into food baits are applied at the resting and feeding sites of the fruit fly (spot spray), instead of spraying the whole mango farm (Dekker and Messing, 1999).

1.1 Justification of the study

The decline in production of mango has had serious consequences on the livelihoods of many farmers who depend on mango production for income generation. Many factors hinder the production of mango from achieving its full potential. In Ghana, one of the most notorious insect pests, are the fruit flies - which can cause damage to the fruits leading to about 60-85% losses, depending on crop variety, season and locality (Billah, 2004; Billah *et al*., 2006). Several control measures are available for fruit fly control worldwide, which include insecticide sprays, use of food baits or protein baits, Male Annihilation Techniques (MAT) and use of entomopathogenic fungi. The main control strategy previously used by farmers in fruit fly control was the use of synthetic insecticides (White and Elson-Harris, 1991; Afreh-Nuamah, 2007). The chemicals used by farmers are mainly carbamates and organophosphates which are expensive and cannot be afforded by many farmers. This type of control also has serious environmental consequences, increases production costs and further causes reduction in the profit margin of growers. This action can also result in high levels of pesticide residues in harvested fruits.

Currently, global efforts toward fruit fly control include the use of food baits (Billah *et al*., 2010a & b; Ekesi and Mohamed, 2010; Billah & Wilson, 2016). Food baits are attractants that are neither sex- nor species- specific (Epsky *et al*., 2014). The product is a mixture of food substances and insecticides that attract and kill fruit flies on consumption of the product. However, increasing cost of these commercial baits has made it inaccessible to farmers in many parts of the world, most especially in the developing countries, including Ghana. For example, as a liquid product, the weight adds additional dimension to its importation, and makes the unit cost of the product very expensive. Small-scale farmers cannot afford these food baits and in most cases do not carry out any form of food bait control in their farms. Large proportions of yields are lost annually due to fruit fly damage from not using food baits. Aside from the cost of importation of

these baits, clearance and delay at the ports are some of the major reasons that prevent the local farmers from getting access to these protein baits. To circumvent these problems, evaluation and improvement of the effectiveness of locally produced baits may provide alternatives for small-scale and mid-scale growers to improve crop protection, and for fruit fly population suppression (Epsky *et al*., 2014). Producing protein baits from local materials can totally reduce the cost of baits, and the inconvenience of importation could be adequately dealt with. The use of waste brewery yeast has led to successful development and use of attractive protein baits like NuLure, Solbait and Corn Steepwater (US), Promar (Malaysia), Royal Tongalure (Tonga) (Ekesi, 2010; Billah, 2016). Promar, has been successfully used to control large populations of fruit flies in Carambola Plantations in Malaysia (ACIAR, 2014). Field experiment conducted in Fiji using protein baits sprays indicated that these locally produced protein baits could be used to effectively control fruit flies in both commercial and wild stands of guava (Australian Centre for International Agricultural Research, 2014).Countries that have developed their own products indicate the costs involved are much lower than their imported alternatives. In Ghana, scientists have prepared protein baits from brewery yeast waste (wastes from Beer Brewing Companies) Eg. (Banini, Bulley, Billah *et al*., 2010ab; Billah, 2014; Billah & Wilson, 2016) as it has been done in several parts of the world including Australia and Mauritius where yeast autolysate from brewery yeast waste are being used for protein baits preparation with great success (Chinajariwong *et al*., 2003; Sookar *et al*., 2003). However, preparing protein baits from waste brewery yeast involves a series of complicated processes. For example, the waste brewery yeast are heated, followed by a series of systems to breakdown the proteins by enzyme to release the volatiles attractive to the insects. The total cost of the proteolytic enzyme required for the proteolysis is very high. This cannot be afforded by many poor and local mango farmers. Also, the processes involved in getting access to the waste brewers yeast from Beer Brewing

Companies are very cumbersome and the locations are far away from majority of local mango growers in Ghana.

In Ghana, there are a number of local drinks which are brewed with a lot of brewing wastes, which are readily available for limited use as feed in the poultry and livestock industry. This study seeks to put these wastes to good use in fruit fly control, because any fruit fly intervention for farmers must be readily available, affordable and acceptable to low-income farmers, who constitute the majority of mango producers. This study therefore will evaluate the substitution of commercial components with cheaper, home-made ones in reducing crop losses as well as the costs of controlling fruit flies. The findings would provide products that could be found in local markets, to help small scale farmers reduce cost and losses due to fruit flies in Ghana.

1.2 General Objectives

To assess the performance of two local waste brewery materials for use as protein baits in the management of fruit flies

Specific objectives

1. To assess the efficacy of Spent Brewery Grain of Pito and Brukutu against fruit flies.
2. To compare the performance of two locally-available spent products (Pito and Brukutu) with the two standard products - SUCCESS Appat (GF-120) and Great Fruit Fly Bait (GFFB)
3. To determine infestation levels of fruits from the different treatment plots.
4. To assess species composition of fruit flies in mango farms.
5. To determine relative fly densities in the mango farm.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The mango crop

2.1.1 Origin, botany and description

Mango (*Magnifera indica*) is a member of the Anacardiaceae family. The mango tree is believed to have evolved as a canopy layer in the tropical rainforest of Southeast Asia, specifically Eastern India, Burma and the Andaman Islands where it has been cultivated for years (Medina *et al*., 2002). Reference to mangoes as the ‘‘food of the gods’’ can be found in the Hindu Vedas. The name of the fruit came from the Tamil word *maangai* or the Malayam word *maanga* and popularized by the Portuguese after their Indian exploration, hence the word *manga* in Portuguese (Pradeepkumar *et al*., 2008). The Portuguese transported the mango from India to West Africa in the early 16th century (Medina *et al*., 2002). It is considered to be “the king of all fruits” for its delicious taste, attractive nature and strong aroma (Salunke and Desai, 1986; NICRA, 2012). Mature mango trees attain a height of up to 30 meters and can survive well for more than one hundred years (Kaur *et al*., 1980). Mango can grow well and develop in areas with a minimum annual rainfall of 1000mm, and can withstand dry periods for months (Akurugu, 2011). An average minimum and maximum temperature of between 24 ^{o}C to 30 ^{o}C are required for the production of mango fruit (Akurugu, 2011). It can also grow well in the warm climates of the tropical (Braimah *et al*., 2004) and subtropical regions of the world (Bally, 2006) at elevations from sea level to about 1200m above sea level (Bally, 2006). It is evergreen tree with alternate, spirally oblong ovate leaf arrangement. The older leaves reaches lengths of 12-15cm long. Mango grows in a slightly acidic (*pH* = 5.5-7.5) and well-drained soil, whether it is sandy, loam or clay. In some case it can grow in alkaline soil (Kadman *et al*., 1960). Mango is also drought-tolerant, and can withstand occasional flooding (Singh, 1960). A good timely rainfall is necessary for best fruit and flowers to set up rather than the total rainfall. The inflorescence is erect and widely

branched with hundreds of small flowers. The flowers are pink to red in colour and 6-8 mm in diameter. Both female and male flowers are found within a single inflorescence. Pollination in mango flower is done by insects, in particular bees (Singh *et al.*, 1962; Jiron and Hedström, 1985). The fruit is a drupe of variable size and shape, ranging in weight from a few grammes to more than 1kg. It is pulpy flattened, rounded or elongated in shape (Pope, 1929). It has a fairly fine skin covered with lenticels. Its colour varies depending on the variety, from green, through yellow, orange to reddish-purplish. At maturity, the pulp turns yellowish-orange and usually firm, but mostly juicy when ripe. Mango fruits vary in their degree of fibrousness depending on variety. Where fibres occur they are mostly around the stone to a greater or lesser degree depending on the variety (Litz, 1997).

2.1.2 Distribution of mango

According to Morton (1987), Indian traders and Buddhist priests introduced the mango into Malaysia and the Philippines in the 4th and 5th Centuries B.C. The mango was carried to East Indies about the 10th Century A.D. by the Persians. The earliest visit of the Portuguese transported the mango from India to East Africa and Brazil. The Spanish traders took the mango from the Philippines to the West Coast of Mexico and then to Hawaii (Nakasone and Paul, 1998). Mango is now found in all tropical areas as well as many sub-tropical regions of the world. Mango exists in two types, one from India and other from the South-East Asia. These two types of mango were brought into Florida a century ago where they produced considerable progeny either by natural or artificial hybridization for commercial purposes. This area is considered to be a center of secondary diversification, and most of the varieties found on the export market are derived from these hybridizations (Litz, 1997).

2.2 Introduction of Mangoes into Ghana

The Turpentine and Jaffna cultivars were the first to be introduced into the country by the early Portuguese missionaries. The Turpentine quickly established and spread throughout the country. It is now referred to as the local cultivar. The missionaries grew Jaffna mangoes around their bungalows and referred to them as Ceylon (now Sri Lanka) mangoes. The exotic varieties of the crop grown in Ghana include: ”Kent”, “Keitt”, “Palmer”, “Haden”, “Tommy-Atkins”, “Irwin”, “Sensation”, “Julie”), which were introduced from Florida to Ghana nearly 40 years ago (Campbell *et al*., 2002).

2.2.1 Mango Varieties Propagated in Ghana

Features such as agronomic properties, resistance to various pests, storage and transportation capabilities and market niche among others are considered for choosing mango varieties for planting. Currently, mango varieties grown in Ghana include: ”Kent”, “Keitt”, “Palmer”, “Haden”, “Tommy-Atkins”, “Irwin”, “Sensation”, “Julie”) and the local variety (GEPC, 2005). Yilo Krobo District in the Eastern region and Shai Osudoku District in the Greater Accra has about 16 varieties of mango of which the ‘Keitt’ and ‘Kent’ are of economic importance, followed by the ‘Haden’, ‘Palmer’ and the ‘Julie’ in order of importance **(**Odzeyem, 2007**)**. The most popular commercial varieties for export market are the Keitt and Kent (GEPC, 2005).

Figure 2.1 Keitt and kent mango

Keitt variety Source: www.specialtyproduce.com Kent variety Source: www.amazon.com

2.2.2 Description of some mango cultivars in Ghana

2.2.2.1 Keitt

It produces a medium size and moderately vigorous tree. The tree tends to be upright with an open canopy. Typical Keitt fruit weighs between 510 and 2000g. The fruit is normally oval with a rounded broad base measuring 13-15cm long by 9-11cm broad by 8.5-10cm thick. The fruit skin is tough, thick and the pulp is juicy and firm, with little fibre. Seeds are mono-embryonic and housed in thick and woody stone. It is a late-season cultivar and quite tolerant to anthracnose disease, packing and shipping stress (Campbell *et al*., 2002). It is an Indian strain thought to have originated in Florida in 1945, and introduced into Africa and Ghana (Vanniere *et al*., 2011).

2.2.2.2 Kent

The tree is large and vigorous with dense upright canopy. Typical Kent weighs between 600 and 700g. The fruit is oval shaped with a rounded base and measures 11-13cm long, 9.5-11.0 cm broad and 9-9.5cm thick. The fruit is tough, thick and adherent to the pulp. The pulp colour ranges from orange to yellow to deep yellow and has a sweet rich flavour and pleasant aroma. Seeds are mono-embryonic and housed in thick and woody stone (Campbell *et al*., 2002). The Kent variety was developed in Florida in 1944, and it is a direct descendent of the Brooks cultivar which was derived from the Sandersha seedlings.

2.2.2.3 Palmer

It is moderately vigorous tree, forming an upright, large and tight canopy. The fruits measure 8.5-10 cm broad by 6.5-7.5 cm thick by 12-15 cm long and weigh between 510 to 850g. The fruit is oblong with a rounded base. Seeds are mono-embryonic and encased in a medium-thick in thick woody stone (Campbell *et al*., 2002). It is susceptible to anthracnose and highly sensitive to cold.

2.2.2.4 Tommy Atkins

This variety was developed and grown for commercial export in Florida in the early 1920's. It is a vigorous tree with dense rounded canopies. The skin of the fruit is tough, thick and adherent. The fruit weighs between 450 and 700g. They are oval to oblong with broadly rounded bases. They measure 12-14.5cm long, 10-13cm broad and 8.5-10cm thick in dimension. The pulp is juicy and firm with medium amount of fibre. Seeds are mono-embryonic and housed in thick and woody stone (Campbell *et al*., 2002). They are known to be resistant to anthracnose disease as well as handling and shipping stresses.

2.3 Commercial production of mango in Ghana

Mango is not indigenous to Ghana but mango trees are found all over Ghana. Commercial production of mango are mainly found in two distinct agro ecological zones (the savannah and transitional) which constitute Northern Ghana, around Tamale and Southern Ghana covering Greater Accra, parts of the Eastern and the Volta regions. These areas with abundant moisture and hot temperatures are suitable for large scale production of the fruit. It grows best in areas of moderate rainfall and high light intensity, so the savannah areas are the best for mango (GNA, 2005; MOFA Report, 2007; GNA, 2008;). In the Northern Ghana, the harvest season runs from March (early varieties like 'Irwin' and 'Haden') to June (late varieties like 'Keit' and 'Kent'). There are two harvest seasons in the Southern Ghana with the main season running from mid-May to July, and the minor season in December and January. The crop is cultivated locally by both small-scale farmers and medium to large-scale holder (Akurugu, 2011). Mango production in the Northern Savannah Zone is fast expanding due to the support provided by the Integrated Tamale Fruit Company (ITFC).

2.4 Economic importance of mango

The importance of mango to many Ghanaian has made it to be described as, 'Golden tree', 'Next cash crop' and 'Ghana's future,' among others (Avah *et al*., 2008). Mango production can help in the alleviation of poverty and improve the economy through export earnings and was predicted as Ghana's untapped 'Gold Mine'. Statistically, it is estimated that mango export earnings shows that the crop has the potential to transform the country's economy better than cocoa and other traditional export crops (GNA, 2008). It is being advocated that value addition to the fruit as concentrate, juice and pulp would go a long way to increase the value of fresh fruit in stocks. Since it ripens at the end of the dry season and at the start of the rainy season, the mango is a basic source of nutrition for rural populations living in the Sudano-Sahelian regions of West Africa. Mango is one of the fruits with a very high nutritional content. It contains carbohydrates, protein, minerals (calcium, iron, zinc, copper, Magnesium potassium, phosphorus, sodium, manganese, and selenium), fats and vitamins especially vitamins A (beta carotene), B_1, B_2, C or ascorbic. The vitamin C content decrease as the fruit ripens, giving way to increased concentration of glucose and sucrose (Bally, 2006). Ripe pulp of mango provides 74 Kcal of energy per 100g of edible portion; and has a moisture content of 79.2-82.0%, total soluble solids of 0.49-0.58% and crude protein content of 0.38-0.62%, with respect to fresh fruit weight (Hussain *et al*., 2002). According to the European Union Strategic Marketing Guide (EUSMG, 2001), favourable climatic conditions and low labour cost leading to the low production cost give the South American and African countries a strong position on the European markets. The report further stated that Ghana compared to some of the countries in the southern region is closer to Europe and thus gives it the urge in terms of market opportunities due to lower transportation cost and shorter delivery times. Irrespective of these opportunities, Ghana is unable to take advantage due to the uncompetitive state of the industry.

2.4.1 Uses of mango

According to Medina *et al*.(2002) mango is regarded as one of the most recommended fruits with medicinal importance to fight beriberi, heal bronchial diseases and cure brain fatigue, insomnia, wrestle heart burn and mental depression. Some volume of the mango juice are processed for preservation into various products such as fruit salads, puree, jams canned sliced frozen mango (GEPC, 2005). The kernel contains 8-10% good quality fat which is used for making soap and also as substitute for cola in confectionery. The timber is used for making boats, furniture and flooring. Mango leaves are used to decorate the entrance of a household amongst Hindus (Pradeepkumar *et al*., 2008).

2.5 Production constraints of mango in Ghana

Several factors, affect mango production from achieving its full potential. Some of the factors include irrigation and storage facilities, lack of micro-credit to farmers and out growers, insufficient investments, limited extension services to mango farmers, inadequate basic and adaptive research as well as major pests and diseases problems (Norman, 2003; Jaeger, 2008). The USAID-commissioned global horticulture assessment identified the following primary issues as of core importance to the development of the horticulture industry in producer countries: (1) market systems, (2) postharvest systems and food safety, (3) sustainable production systems and natural resource management, and (4) nutrition and human health (World Bank, 2010). A critical look at the situation in Ghana shows a similar trend, and within the constraint of sustainable production systems, biotic stresses that include pests and diseases are considered crucial to development.

Mango, like most other tropical crops is attacked by several pests. Some of these pests are important in every part of the world, especially where they are of quarantine importance. The

relative importance of any one of them varies from region to region. The most important common pests of mango across the world are mealybugs, stone weevil, fruit flies, scale insects, mites, thrips and fruit borers. The mango stone weevil, *Sternochetus mangiferae* (Coleoptera: Curculionidae) is another major pest of quarantine importance. Currently however, infestation and damage by fruit flies have been the key biotic constraint in the country (PPRSD, 2010). There are more than 4000 species of fruit fly worldwide, with a number of them recorded in Ghana as pests of economic importance (Billah *et al*., 2009).

2.6 The fruit fly

2.6.1 Classification and Description

Fruit flies belong to the Order Diptera and sub-order Cyclorrhapha. The term "fruit flies" is used for two distantly related groups of flies, the families Drosophilidae and Tephritidae. The Drosophilidae includes "flies", which in reality, are micro-fungi feeders and have acquired this name because of their habit of feeding in decaying fruit (Ekesi and Billah, 2006; 2009). "True fruit flies" belong to the family Tephritidae because most species attack living plant material, and an estimated 40% of the over 5,000 described species attack intact and growing fruits. The family includes some of the most serious fruit pests such as *Zeugodacus cucurbitae* (Coquillett), *Bactrocera latifrons* (Hendel), and *Bactrocera carambolae* Drew and Hancock. The ovipositor of the females of fruit flies looks similar to the "sting" of a wasp, with which they puncture the skin of healthy fruits and lay their eggs in there. Larval development is completed within the fruit (which may become rotten as a result) and the fully grown larvae then drop into the soil and form a puparium (Ekesi and Billah, 2009). In order to distinguish them from the Drosophilidae, the Tephritidae are characterized by an elaborate wing patterns and the possession of a telescopic ovipositor by the females (Ekesi and Billah, 2006; 2009). Sub-Saharan Africa is a home to about 915 fruit fly species from 148 genera, with 299 species developing in either wild or cultivated

hosts, or in both (Ekesi and Billah, 2006; 2009). The indigenous fruit flies in Africa mainly belong to four genera: *Bactrocera, Ceratitis* McLeay, *Dacus* and *Trirhithrum* (White and Elson – Harris, 1992; Thompson, 1998). For example, *C. capitata* (Wiedemann), *C. fasciventris* Bezzi, *C. rosa* Karsch, *C. cosyra* (Walker), *C. anonae* Graham and for *Dacus* species, *D. vertebratus* Bezzi, *D. bivittatus* (Bigot), *D. cilliatus* Loew, *D. punctatifrons* Karsch and *D. frontalis* Becker (Ekesi and Billah, 2006). Most of them feed on a range of wide crops and their host range overlaps to varying degrees. Several representatives are known to attack different types of commercial and wild fruits and vegetables, causing considerable damage to horticultural crops (Ekesi and Billah, 2006; 2009).

2.6.2 Biology of the mango fruit fly

Fruit flies are multi-voltine species (that is they produce several generations per year), and inhabit tropical and subtropical regions without undergoing a developmental pause. To manage fruit flies in orchards, there are several important things to understand about their biology so as to effectively target and control the pest (Ekesi and Billah, 2006; 2007). Mango fruit flies vary in species and climatic requirement, but they all go through similar life cycle. They undergo complete metamorphosis (eggs, larvae, puparia, and emerged adult flies). There are slight differences in terms of the number of egg laid, larval and pupal durations, adult sexual maturity period from emergence and life span. The generalized life cycle of mango fruit flies are shown in Fig 2.2

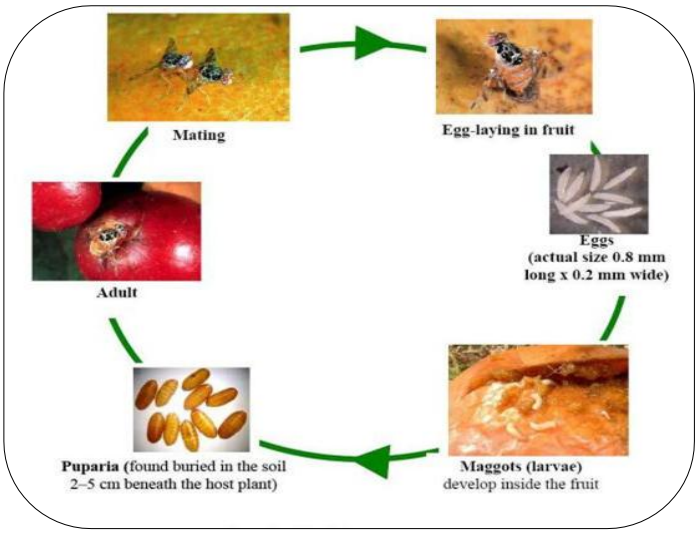


Figure 2.2: Life cycle and biological destruction caused by fruit flies (Source: Ekesi & Billah, 2007)

2.6.2.1 Egg stage

Adult flies become sexually matured 4 to 10 days after emergence and begin to mate soon after. Female tephritids are characterized by a long, extensible ovipositor which allows them to insert their egg beneath the skin of suitable hosts, especially in ripening or ripe fruits and vegetable to a depth of about 2-5mm (Ekesi and Billah, 2006; 2007). One female mango fruit fly can lay between 400-1000 eggs in 24 hours (FAO/AusAID/UNDP/SPC, 1997). The eggs are banana-shaped and are laid in batches of 3-8 depending on the species, although some species lay single eggs. The egg is usually glistering white to yellow, becoming slighter darker towards the time of hatching. The shape and size vary according to the species (White and Elson- Harris, 1992). The eggs of *Ceratitis capitata* are elongated and gently tapering. During oviposition, saprophagous bacteria from the intestinal flora of the fly are introduced into the fruit, causing rot of the fruit tissues surrounding the eggs. These bacteria cause fruit decay, providing a medium in which the larvae feed. The eggs are hatched after several days (3-12 days), depending on the temperature and the physiological stage of the host fruit into larvae.

2.6.2.2 Larval stage

The larvae are creamy-white maggot, which derive its food from the fruit that has started to decay due to bacterial infection. The larval feeding makes the fruit inedible and in some cases, cause premature fall of the fruit. The larvae grow in size by shedding its skin (molting) twice, through three larval stages. The first instar larvae are completed before emerging from the eggs in some *Urophora* species (White and Kormeyev, 1989). The first instar larvae are extremely small almost translucent, with little surface sculpturing. Second instar larvae are creamy-white (some may by discoloured due to gut contents) and very similar though smaller than the third instar of some species (White and Elson-Harris, 1992). The third instar larvae tend to be cylindrical and rounded almost truncated at both ends of the body and are able to hop and flip to leave the fruit while still hanging on the tree or fallen (Ekesi, 2006). The larva is fully grown in about 10.5 days after hatching measures about 7-8mm long when the average temperature is 25-26 ^{o}C (Ekesi and Billah, 2007). After this point, the larvae drops to the ground burrows for a short distance into the soil or debris and transforms into a brown, white or black 4-12mm long pupa in puparium that is a hard case in which the larvae becomes adult (Ekesi and Billah, 2007).

2.6.2.3 Pupa stage

The puparia are found buried in the soil 2-5cm within the canopy line beneath the host plant. Depending on the climatic condition the adult emerges from the pupal case after 10 to 20 days. When pupariation is complete, a winged fly emerges and digs its way out of the soil or the debris (Ekesi and Billah, 2007).

2.6.2.4 Adult stage

The newly emerged adults are not sexually matured (Steck, 1984). They become sexually mature 4 days after emergence, and copulation has been observed 5 days after emergence (Ekesi and

Billah, 2007). Both sexes are sexually active throughout the day. When the daily mean temperature averages from 24-26 ^{o}C most females are ready to mate from 6-8days after eclosion (Steck, 1984). Adult flies feed on various kinds of food, such as glandular secretion of plants, flower nectar, plant sap, rottened fruits, bird's feaces, and honeydew secreted by homopterous insects. The population reduces to minimal for 3-4 months when there is lack of fruit. Adults rest in shady locations unless feeding, mating or laying eggs.

2.7 Economic importance and damage of fruit flies

About 70 species of fruit flies are considered important agricultural pests and many others are minor or potential pests (White and Elson-Harris, 1992). Citrus, mango, apples and many others are the most important crops attacked by fruit fly species. Some seed crops such as sunflower and safflower are also affected (White and Elson-Harris, 1992). Because of the polyphagous habits of their larvae, many species of Tephritidae inflict heavy losses on fruit and vegetables crops. Economic effects of pests species include not only direct loss of yield and increased control costs but also the loss of export markets and or the cost constructing and maintaining fruit treatment and eradication facilities (White and Elson-Harris, 1992). Direct fruit damage occurs when adult female fly punctures the fruit skin and lays eggs underneath it. Damage symptoms vary depending on the host fruit species. During oviposition, saprophagous bacteria from the intestinal flora of the fly are introduced into the fruit, causing rot of the fruit tissues surrounding the eggs. When eggs hatch, the rotten fruit tissues make it easier for the larvae to feed. The puncture and feeding galleries made by developing larvae also provide access for pathogens to develop, and increase the fruit decay, and thus, rendering it unmarketable. Generally, the fruit falls to the ground, the larvae move out of the fruit and pupate in the soil (Billah, 2004; Ekesi, 2006). Indirect fruit damage and losses result from quarantine restrictions that are imposed by importing countries to prevent entry and establishment of such unwanted pest species through the border lines. Lux *et*

al.(2003a) estimated that out of 1.9 million tonnes of mangoes produced in Africa annually, about 40% is lost due to fruit flies. Infestation rates vary among countries and seasons ranging from 5% to 100%. In Benin, between the months of April and June the damage assessment of mango, showed losses varying between 10-57% (Vayssières *et al*., 2005). Mango production and export in Senegal is threatened mainly by one species of fruit fly: *Bactrocera dorsalis*. Losses range from 50% - 85% and in extreme cases reaching 100% in the Bas Saloum region (IMO, 2011). Due to high infestation of fruit flies in Cote d'Ivoire, harvest for export becomes uneconomic and as a result of this mangoes collected were used exclusively for the local market. As a result, incomes were affected and many farmers moved away to other perennial cash crops like cashew nuts (Hala *et al*., 2006).

Production of mango in Ghana is seriously affected by fruit flies. About half a million dollars were attributed to fruit fly losses in the agricultural industry. *Bactrocera dorsalis* and *Ceratitis* species were fruit fly recorded in 2009 as the most important pest species of the fruit fly (Ghana Report, 2010), causing over 65% of the damage (WAFFI, 2010). *Dacus*, *Ceratitis* and *Trirhithrum* species were the fruit fly pests that were seriously causing damage to mango in Ghana before the invasion of *B. dorsalis*. Twenty-five (25) metric tonnes of mangoes from Ghana were intercepted in France in 2007 as a result of *B. dorsalis* (Stonehouse *et al*., 2008). Today the *B. dorsalis* has taken over the indigenous species in most mango farms. Billah *et al*. (2006) conducted a survey in three major fruit growing and trading regions in Ghana, and detected *B. dorsalis* in 29 out of 37 localities surveyed.

Indirect losses resulted in quarantine restriction imposed by importing countries to prevent entry and establishment of fruit flies. In 2008, farmers in Malindi, Coast of Kenya lost US$ I million to *B. dorsalis* export market restrictions on mangoes resulting in loss of income to hundreds of

smallholder mango growers and also loss of US$1.9 million from export due to restriction from South Africa (TEAM, 2010). In Mozambique, *Bactrocera dorsalis* was first detected in 2007 in Cuamba district, Niassa province (Correia *et al*., 2008). Due to its detection, quarantine measures were imposed on Mozambique by importing countries of fruit and vegetables produced leading to loss of international markets. The temporary closure of South African market for three weeks in october 2008 resulted in the loss of about 2.5 million U.S. dollars (Cugala, 2011).

2.8 Influence of weather parameters on trap catches of fruit flies

It has been established that before developing pest management programme for a specific agro-ecological system, it is important to have basic information about the pest in relation to weather parameters which will help in determining suitable methods of control and appropriate timing (Lasker and Chatterjee, 2010). Relative humidity, temperature, rainfall, wind velocity and temperature are weather factors that have influence on fly catches. Many insects are attracted and respond to semio-chemicals only at certain times such as dawn, midday, dusk and night (Weinzierl *et al*., 1995). Studies conducted by Vayssières *et al.* (2009a**)** on the influence of humidity, rainfall and temperature indicated that these are closely related with fruit fly distribution and population dynamics. Rainfall and relative humidity have positive relationships with *Bactrocera dorsalis* counts. Wind speed and direction determine the extent of insect movement from surrounding areas to traps within the field.

Temperature is one of the most important environmental factors affecting insect developmental rate, maturation, survival and distribution. There is significant positive correlation between trap catches of *Bactrocera dorsalis* and maximum and minimum temperature (Agawal *et al*., 1995). Minimum temperature and wind speed had a significant positive correlation on traps of *Bactrocera dorsalis* whereas with relative humidity it had negative correlation (Sushilkumar *et*

al., 1997; Sarada *et al*., 2001). Mortality increases when the temperature is outside 18 ^{o}C to 27 ^{o}C (Fletcher, 1987). In fruit flies it is directly related to pupa development time and adult emergence. A laboratory study carried out on the effect of four temperatures on pupal development and sexual maturity of *Anastrepha obligua* adults revealed that: pupal duration decreased with an increase in temperature; constant 18 ^{o}C and 20 ^{o}C resulted in low percentage of pupation; pupal weight loss and less flying ability; lower calls of mating; at temperature of 30 ^{o}C adult had low sexual efficiency, as well as a lower proportion of calls and mating; the fecundity of the female was higher at low temperatures.

2.9 Pest management/control methods used for fruit flies

The two basic approaches in the management of fruit fly are IPM approach and eradication approach (Lux *et al*., 2003a). The IPM aim at controlling fruit fly population in order to reduce yield loses and the eradication aim at targeting area-wide elimination of the fly population to create certified 'fruit fly–free' zone.

2.9.1 The eradication approach

Myers *et al*. (1998) described eradication as the elimination of all individual of the species from a geographic area where reinvasion is likely to occur. Some pests can be controlled by genetic manipulation known as Sterile Insect Techniques (SIT) (IAEA, 2003) through a 'birth control' method. It is a method that can be applied to control species of insect that reproduce by sexual reproduction (Van der Vloedt and Klassen, 2006). It does not release exotic agents to the environment and is species specific (Hendrich and Hendrichs, 1998). The technique is very effective if the sexually sterile males are aggressive and successfully compete with the wild males in searching for and mating with indigenous females. The first sterile programme against Medfly was initiated in Southern Mexico in 1997. The main aim was to prevent the spread of the Medfly

from Central America to Mexico and the USA. The programme succeeded in 1982 in eradicating Medfly from areas it had already infested in Southern Mexico and since then a sterile fly barrier has been maintained (Hendrich and Hendrichs, 1998).

2.9.2 IPM approach

However, when eradication methods are not available or justifiable, the Integrated Pest Management (IPM) strategy is used to allow quality fruit production in the presence of the pest. This strategy combines as many compatible methods as possible to reduce yield losses and allow fruit production (Klungness *et al*., 2005). The major components of such an integrated approach include the use of traps containing para-pheromones or protein bait (Lux *et al.*,2003a), biological control using parasitoid, predators and pathogens, phototoxic dye food baits (Moreno and Mangan, 2002), and cultural practices such as orchard sanitation and fruit bagging (Lux et al., 2003a; Ekesi and Billah, 2006, 2007).

2.9.2.1 Soil inoculation

The use of entomopathogenic fungi to control fruit fly has gain more attention in Africa. In Kenya two applications were evaluated with a number of strains of *Metarhizium anisopliae.* 1) Putting the pathogens in the soil to create a hostile environment for the pupariating and puparia, and 2) use of pathogens as alternatives to pesticides in localized baiting stations as killing agent for the attracting of fruit flies. During application, the pathogen is applied by hand along the drip line of the fruit canopy and raked in the soil (Ouna, 2010). This system was found to be effective against a wide range of fruit fly larvae, pupae and adult of a wide range of fruit flies; *Ceratitis cosyra, C. fasciventris, C. capitata,* C. *rosa* and *C. anonae*. Special attention was given to *Metarhizium anisopliae* as it was able to invade through the cuticle of the insect and did not require any ingestion. Lux *et al.* (2003a) reported that adult emergence from the treated soil in the laboratory

and field cages reduced by 6 to 68%. This was attributed to the inability of germ tubes to penetrate the pupal integument as it hardens due to sclerotization.

2.9.2.2 Post-harvest fruit treatment

Exports of vegetable and fruit crops to European market would be rejected, without post-harvest treatment which ensures that no developmental stages of quarantine insects are present in the fruit. Although there are different postharvest disinfestation techniques, the appropriate quarantine treatment technologies include: i) heat treatment ii) cold treatment and iii) irradiation with gamma particles to kill the developing flies (Robinson, 2005). Airports in France detected the presence of one European and12 non-European species of Tephritidae as pests from tropical fruits imported even after post harvest treatments (Bayart *et al*., 1997).

2.9.2.3 Cultural Control and Orchard sanitation

Normal production systems that are carried out in the farm help to produce quality and healthy fruits and also helps to reduce the pest population of insect. Farm sanitation and crop hygiene are the main methods used in cultural control that focus on the life cycle of the fly, so that the larvae do not grow in the soil. These practices entail the picking of dropped fruits either premature or mature from the ground (Afreh-Nuamah, 1985; 1999; Ekesi and Billah, 2006, 2007; Billah & Wilson, 2016). The fruits are buried at least, 50 cm deep, to prevent the emerging adult flies from reaching the soil surface (Ekesi and Billah, 2006, 2007).

On the other hand, fruits are collected into thick black polythene bags, tied and exposed to the sun for 10-14 days for the larvae in the fruits to be killed as a result of heat generated in the bags (Ekesi and Billah, 2006, 2007). Another way is by digging a pit and pouring all the collected fruit from the soil and the infested fruits on the trees into the pit and sufficient lime added to kill the

larvae. Cultural control for fruit flies is a tedious exercise but can be quite effective if the fruits are regularly collected and destroyed twice a week for the entire season. Collection and destruction of fallen, damaged, over-ripe fruits is strongly recommended to reduce populations of fruit flies in orchards. The collected fruits should be destroyed by either burning or burying. The collection and deposition of fallen, damaged and unwanted fruits in an Augmentorium (plural augmentoria) (Klungness *et al*., 2005) is being strongly advocated among fruit and vegetable growers across Africa. Augmentorium is a tent-like structure designed with a skirt of materials that is buried in the ground and an upper roof (at least 1m above the ground) into which infested fruits are placed in through an opening to sequester and prevent the egress of adult tephritid flies coming from the fruits, but allow parasitoid wasps to escape. Removal of infested fruits can contribute significantly to reduction of fruit fly population (Rwomushana, 2008). Other cultural practices which are carried out in the farms include: avoiding the growing of alternative host of fruit in the orchard, avoiding the growing mango of different varieties with different growth cycles in the same farm, weeding the farm and in some cases infested fruits are collected and used to feed the pigs which helps to reduce the population of larvae in the farm.

2.9.2.4 Mechanical fruit protection

The main aim behind this control method is to prevent the adult female flies from using their ovipositor to puncture the fruits and lay their eggs in the fruits. This is achieved by using paper to wrap all the individual fruits in the farm (Ekesi and Billah, 2006, 2007). Fruit must be wrapped well at least, one month before harvest to prevent attack by fruit fly. Although it is time consuming and laborious, it is cheap and environmentally-safe to use (Ekesi and Billah, 2006, Billah *et al.*, 2013). This is normally done in backyard gardens and on small scale for high value crops (Ekesi and Billah, 2006, 2007; Billah *et al*., 2013)). In the continent there are no known published researches on the use of bagging of individual fruits against tephritid pests.

2.9.2.5 Early Harvesting

Fruit fly infestation could be avoided by harvesting fruit and vegetables early when they are not fully mature to prevent attack by fruit fly. Fruit flies developmental stages do not grow in certain fruit such as sapodilla, papaya and banana when they are not fully matured. Only the matured and ripe fruits are good host. Bananas at the mature green stage are not likely to be attacked by fruit flies. Thus, early harvesting is an important technique to prevent fruit fly infestations in the production of these fruits (Afreh-Nuamah, 1985; Ekesi and Billah, 2006). After harvesting, fruits that have been missed on trees should be collected, used or promptly destroyed (Afreh-Nuamah, 1985).

2.9.2.6 Biological Control

Predators and parasitoid used for suppressing fruit flies has gain more attention in fruit fly control because it is permanent, economical and safe (Ekesi and Billah, 2006). In this control method, the natural enemies are allowed to actively comb for the fruit fly and kill the host (Gilmore, 1989).

Oecophylla longinoda (Latreille) (Hymenoptera : Formicidae) protects tree crops as it actively patrols canopies and prey upon or deters a wide range of potential pests. The adult fruit flies are not killed by the ants. The ants rather prevent them from ovipositing and laying their eggs on the fruits. In some cases where oviposition occurs, these ants are known to dig out and feed on larvae that are developing on the fruit. The ants performs two main functions in reducing damage to fruits and pest population as they feed on the larvae even though damage has already been done to the fruit. A study conducted in Benin revealed that fruit damaged were significantly reduced by *Oecophylla longinoda* by deterring fruit flies from attacking and damaging the fruits (Van Mele and Cuc, 2000: Van Mele *et al*., 2007; Ativor et al., 2012). In addition, rodents have also been reported to reduce the number of larval population in the field when they feed on the infested fruits (Drew, 1989).

Parasitoids have been exploited for the control of Tephritid fruit flies in Africa. The eggs and larvae of fruit flies are attacked by fruit fly parasitoids that lay their eggs and develop in/on the host, killing the host in the process. *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) was introduced in Hawaii from Malaysia and became the dominant fruit fly parasitoid in Hawaii, causing great reduction in fruit fly populations (Vargas *et al*., 2005). *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) have been shown to be highly efficient in laboratory tests for controlling *Bactrocera dorsalis* and five indigenous fruit fly species - *Ceratitis capitata,* C. *rosa, C. cosyra, C. fasciventris* and *C. anonae,* and have successfully been used in the Pacific region against *Bactrocera dorsalis* and *Ceratitis* species (Ekesi and Billah, 2006).

2.9.2.7 Host-plant resistance

Host-plant resistance is a peculiar parameter in Integrated Pest Management programmes. This is done not to cause any harmful effects to the environment, and no extra cost is incurred to the

farmer. Unfortunately, success in developing high yielding and fruit fly resistant varieties have been limited (Dhillon *et al.*, 2005). Some plants may reduce growth, inhibit reproduction, alter physiological, delay and induce various physical or behavioural abnormalities in phytophagous insect (Dent, 1991).

2.9.2.8 Chemical control

Throughout the world the use of direct and blanket insecticide usage is not accepted in the management of fruit fly. The use of some insecticides cannot be avoided and careful chemical choice and application can reduce the hazards caused to the environment. The use of particular insecticides to control an insect requires a thorough understanding of the pest's field biology (Gullan and Cranston, 1994). Agarwal *et al*.(1987) reported that Malathion, dichlorvos and endosulfan are moderately used effectively against the melon fruit fly.

Abdullahi *et al*. (2011) tested a selected few of the most commonly used insecticides in Ghana, and concluded that pyrinix at different rates was best for *Bactrocera dorsalis* control. Cheng *et al*. (2009) tested some insecticidal activity of basil oil, estragole, trans-anethole and linalol on adult of *Zeugodacus cucurbitae*, *Ceratitis capitata* and *Bactrocera dorsalis*, and concluded that all chemicals act faster in controlling them. Chemicals extracted from organic materials such as neem are also used in control of fruit fly. Neem works by alteration of behavior leading to repellent and or anti-feeder effects, disruption of insect development by inhibiting the release of prothoracicotropic hormones and allatotropins (Mordue *et al.*, 1986; James, 2003). Azadirachtin, the active ingredient in neem has minimal toxicity to beneficial organisms such as parasitoids, predators and pollinator (Raguraman and Singh, 1999) and can degrade easily in the environment. Neem Seed Water Extracts were found to be very effective in reducing damage caused by fruit flies (Akosten-Mensah, 1999).

2.9.2.9 Monitoring with attractants

Pest management decisions can be made by monitoring with attractant which deals with understanding insect activity, the peak period of pest activity and seasonal population fluctuation forms important parameters of any pest management strategy. This will help to determine the time of pest outbreak in order to carry out effective control measures (Ekesi *et al*., 2007). Monitoring of fruit fly helps to determine distribution of pest species, determine fruit fly pests in an area, track changes in population levels, determine local hot spots with high populations, for detecting new fruit fly pests in a region and determine the efficacy of control measures (Ekesi and Billah, 2006). Attractants, traps and insecticides are the tools used for monitoring of fruit flies. According to Ekesi and Billah (2006) fruit fly monitoring consists of two types of attractants which include food baits and para-pheromones (male lures).

2.9.2.10 Use of Parapheromones

Parapheromones are species specific lures that are attractive to only male fruit flies from a long distance (White and Elson-Harris, 1992).The aim of Male Annihilation Technique (MAT) is to cut down male fly population to levels that mating occurs at very low levels or does not occur. Male lures in liquid form last between 2-4 weeks (Ekesi and Billah, 2006). The minimum intervals between traps are 30-50m (Ekesi and Billah, 2006).Cue lure (CUE), Trimedlure (TML), Terpinyl acetate (TA), Vertlure (VL) and Methyl eugenol (ME) are the major fruit fly attractants used for monitoring. TML and TA lure several species of *Ceratitis*, ME lures several species of *Bactrocera*, CUE lures several species of *Zeugodacus*, while VL are attractive to *Dacus* species of fruit fly (IAEA, 2003; Manrakhan, 2006). Methyl eugenol is a naturally occurring compound and can be obtained from at least 10 different plant families (Ekesi and Billah, 2006). Ghana is among the countries in Africa where these para-pheromones are currently being used in the management fruit fly (Billah *et al*., 2006; Ekesi and Billah, 2006). Increasing the number of traps

baited with methyl eugenol significantly decreased damage caused by fruit flies (Qureshi *et al*., 1981). Methyl eugenol strongly attracts *Bactrocera dorsalis* and it is technically possible to suppress it using the MAT, as has been achieved in other tropical regions. ME can lure fruit flies from a distance of 800meters as a result of its having both olfactory as well as phago-stimulatory action (Roomi *et al*., 1993). A research conducted by Bagle and Prasad(1983) using methyl eugenol as a bait showed that during March, April May and June recorded mean monthly catches of 1268, 270, 416 and 487 flies, respectively at the peak period activity of *Bactrocera dorsalis*. The same methyl eugenol used in Southern Taiwan by Chiu and Chu (1986) indicated a peak activity of *Bactocera dorsalis* from June to September and increase in population observed during April declined from December to March. TML attract males of *Ceratitis fasciventris* and *C. rosa*. The males of *C. anonae* also respond well to TML. TML does not occur naturally but is a synthetic compound. Males of *Ceratitis capitata* respond well to TML and to a certain extent also TA (Lux *et al*., 2003a). Alpha, 4-trimethyl-3-cyclohexene-1-methanol is the active ingredient in TA. Studies carried out in Cote d'Ivoire from 2000-2002 indicated that *C. cosyra* is most attracted to TA (99.18%), while *C. bremii* is more attracted to ME (Hala *et al*., 2006). TA occurs naturally as an ester. The active ingredient in CUE is 4-(p-hydroxyphenyl-2-butanone acetate) and it attracts males of *Bactrocera* and *Dacus* species. It does not occur in nature, but the most closely related analogue is raspberry. The trapping procedure and traps adopted for fruit flies monitoring are dependent upon the nature of the area and of the attractant (IAEA, 2003).

2.9.2.11 Use of Food baits

Presently, the intensive use of insecticides is being discouraged as a result of their negative impact on human health and environment. Environmentally-safe pesticides and appropriate protein sources (Peck and McQuate, 2000) are used as baits as spot spray or in traps to reduce fruit fly populations below the economic threshold. Food baits (ammonium mimics or their hydrolyzed proteins) mixed with a killing agent are used to suppress fruit fly population and this

has been tested in fruit fly suppression for the management of *Bactrocera* and other species. Food baits are not species-specific and attract both male and female fruit fly (White and Elson-Harris, 1992). IAEA (2007) and Heath *et al*. (2009) recognized the need to develop improved lures and "attract-and-kill" devices for successful fruit fly control. These baits can also attract a number of non-target insects, including beneficial ones. They are available in liquid form. The principal component emanating from the food baits is ammonia. There are varieties of commercially available food baits. These include liquid ammonium salts, yeast products, the three-component lure (consisting of Putrescine, Trimethylamine and Ammonium acetate, PTA) and protein hydrolysates (Ekesi and Billah, 2006). A number of commercial baits are now available in the market; such products as Nulure, Buminal and Sol Bait that can be mixed with insecticides such as spinosad for direct application. Another commercial product is GF-120 (SUCCESS Appat) which is already premixed with spinosad and can be applied using the label information on the packaging container. The field longevity of protein hydrolysate, ammonium salts and yeast products is between 1-2 weeks, while the three-component lures can last between 4-6 weeks. The type of trap depends on the nature of the attractant (IAEA, 2003). During trapping the attractant is mixed with an insecticide so that as the lure brings the insect to the trap, the insecticides kill the insects.

2.9.2.12 The use of SUCCESS APPAT® (GF-120)

It is a combination of spinosad insecticide, sugar, protein, feeding stimulant and attractant design for controlling a wide range of fruit fly population (Moreno and Mangan, 2002). The bait was formulated to attract different fruit fly populations and to use the minimum concentration of an environmentally compatible toxicant for ultra-low volume (2-4 L/ha) application. The trade names are TRACER™, SPINTOR™, LASER™, SUCCESS™, and ENTRUST™ .

The bait is applied as a splash onto the lower surface of the leaves throughout the farm. Component such as protein and sugar in the baits attract both male and female fruit fly. The insecticide in the bait kills the flies by stomach poison as they feed on the sugar and the protein in it. Fruit flies are very active in the morning so they are able to kill the insect when applied at that time. The baits attract fruit flies from several metres away. The bait should be applied again if there is rain after spraying. Spinosad the killing agent in the GF-120 is a combination of spinoyn A and D compounds that are purified from the soil actinomycete, *Saccharopolyspora spinosa* Mertz (Thompson *et al*., 2000) and has low mammalian and environmental toxicity (Dow, 1994). The spinosyn has low contact toxicity to both vertebrates and invertebrates and the active ingredient has to be consumed before they can cause toxicity. In Benin, during 2006 and 2007 a weekly treatment of GF-120 on mango farm resulted in 80% reduction of fruit flies damage when compared to the control farms (Vayssières *et al*., 2009b). In 2007, Smith and Gutierrez (2008) also evaluated the use of GF-120 Bait for the control of Cherry Fruit Fly. The full application of GF-120 greatly reduced but did not completely control Cherry Fruit Fly infestation on sites with high numbers of adults emerging during the first season of treatments. In Senegal, 83% reduction of fruit damage was achieved compared to the untreated mango farms when used in combination with male annihilation (Mbaye *et al*., 2008). However, GF-120 Naturalyte ™ is only efficient against fruit flies feeding (Prokopy *et al*., 2005), and only if used continuously throughout the time of fruiting and post-fruiting seasons (Revis *et al*., 2004).

SUCCESS® Appat is an important food bait for controlling fruit flies. For example, an invasive species, Oriental fruit fly, is very aggressive in attacking nearly 200 plant species. A field study conducted in papaya farms in Hawaii indicate that application of the protein bait GF-120 proved successful at managing oriental fruit flies, *Bactrocera dorsalis* (Pinero *et al*., 2010). Two important parameters of this study were the field sanitation and mass trapping using the male-

specific lure methyl eugenol. Yee and Chapman (2005) also indicated that the SUCCESS® Appat can be used at backyard and abandoned trees, which are heavily infested by fruit flies. When GF-120 was sprayed on single trees to suppress the larval populations, the bait was not able to eliminate the fruit flies after one season, because some flies were not attracted to and did not feed on the GF-120 (Yee and Chapman, 2005; Yee, 2006), or some flies oviposited before ingesting the bait and dying.

The bait is marketed as a concentrate that requires dilution of water before use. The GF-120 formula is registered as organic by US Department of Agriculture and International organic registries (Organic Materials Review Institute, 2002). Field trials in Florida on citrus fruit flies indicate that the SUCCESS® Appat has no effect on honey bees in field application (Rendon *et al*., 2000). Experimental evidence has indicated that GF-120 has minimal or no effect on non-target beneficial insects (Burns *et al*., 2001).

2.9.2.13 The use of Great® Fruit Fly Bait (GFFB**)**

Great® Fruit Fly Bait (GFFB) is a unique product that is formulated based on the knowledge of the biological behaviors and dynamics of fruit flies, especially their needs for special nutrients after emerging from the pupa. It attracts both male and female flies, and particularly immature females searching for protein food so they can develop their eggs (Billah, 2014; Ecoman, 2015). The active ingredient in it is abamectin. Abamectin M is the common name for avermectin B1, a naturally occurring miticide/insecticide, derived from the soil micro-organism, *Strewtomvces avermitilis*. The pesticidal activity of abamectin is related to the interaction with the nerve transmitter, gamma aminobutyric acid.

Ecoman Biotech in Ghana performed their first outreach from December 2013 and February, 2014 for fruit control in order to establish a subsidiary company in Accra, Ghana. These research

were performed in areas where *Bactrocera dorsalis* (Hendel) was causing more than 50% overall damage to the fruits, resulting in heavy losses (Ecoman, 2015). After the trials with GFFB protein bait application, they concluded that, there was significant reduction in fruit damage rate caused by the fruit fly from 30% - 40% to less than 1%, when compared with the same season of the previous year, proving high efficiency and cost-effectiveness. This led to increased wide-scale operation and use of their products.

During January 2015, the Ecoman Biotech implemented an area wide control program in a total area of about 136 acres in Samut Sakhon Province, Thailand. The trial was performed in plots of several crops like – mango, rose apple, guava, grape, lemon, longan, mostly infested by *Bactrocera dorsalis* (Hendel). The results in April, 2015 indicated that average fruit damage in treated plot was 1.96 percent, while in untreated areas, it was 38 percent. The fruit fly damage rate was significantly reduced in plots treated with GFFB (Ecoman, 2015).

Ecoman Biotech led an Area Wide Control Program (AWCP) against Chinese citrus fly, *Bactrocera minax* (Enderlein), the total area of the AWCP was 6,000 Ha. Their report indicated that GFFB achieved high effective fruit fly control in reducing damage rates to crops and resulting in increased yield of 7.6 tons per ha, valued approximately at 2,300 USD/ha (Ecoman, 2015).

2.9.2.14 Spent Brewer's Grain (SBG)

The brewing industry generates relatively large amounts of by-products and wastes; spent hops, yeast and grain. Spent grain is the most abundant brewing by-product, corresponding to around 85% of total by-products generated (Tang *et al.*, 2009). After the brewing processes, the solid residue remaining constitutes the Spent Brewer's Grain (SBG). Traditionally, this SBG is either

sold as animal feed or discarded into streams and other water bodies. The production process is time consuming, complex and sometimes carried out under unhygienic conditions. The grains are steeped in water for 5-6 hours. The grains are then poured in a basket to drain off the excess water without necessarily washing it. They are then spread on mats or in aluminum basins in a cool dry area to germinate. They are turned over intermittently to enhance the germination process. The germination lasts between 3-5 days depending on the humidity. The fresh malt is milled to obtain the flour. The flour is mixed with water in a ratio of about 1: 3 by stirring with the hand or a wooden ladle. The resulting mixture is allowed to settle for 2-3 hours. The supernatant is separated from the sediment and the latter cooked for 1-2 hours after which it is mixed again with the supernatant to give the wort. The wort is allowed to cool, stirred and sieved to constitute the Spent Brewery Grains (SBG). Scientific research has revealed that even though the chemical composition of SBG may be influenced by some extrinsic (the type and quality of secondary raw materials added in the brewing process, harvest time, malting and mashing conditions) or intrinsic (barley variety) factors, always include considerable amounts of essential amino acids, protein and dietary fibre as well as appreciable levels of vitamins, polyphenols, lipids and minerals (Santos *et al*., 2003, Mussatto *et al*., 2006).

Also, the yeast in brewery waste has the potential to be developed into suitable locally-produced baits. Lloyd and Drew (1997) reported that waste brewery yeast is a rich nutritional source of vitamin B-complex, protein (providing all essential amino acids), and minerals which are needed for insect growth and fecundity. Protein used in bait sprays has been tested from several sources. The yeast in brewery waste has been successfully modified into baits for fruit fly control in Queensland, Australia (Lloyd and Drew, 1997). Chinajariyawong *et al*.(2003) evaluated the Australian protein bait, Pinnacle® (Mauri Yeast, Camellia, NSW, Australia) and brewery waste from Thailand, and found that both baits reduced fruit fly infestation significantly when compared

with controls. In Malaysia, a yeast autolysate, Promar, has been used to successfully control large populations of fruit flies in Carambola Plantations (Australian Centre for International Agricultural Research, 2014). It has proven to be an excellent attractant for local species of fruit flies and does not cause phytotoxicity to plant (Vijaysegram, 1989; Loke *et al*., 1992).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites

The study was carried out in two forms: the field and the laboratory. The field work was carried out in the Coastal Savannah agro-ecological zone of Ghana in the Dodowa - Somanya stretch. These two localities are involved in the production of mango and they constitute major mango growing and trading areas in the Southern Ghana. Two mango farms, in each locality were selected at random for the research. Ayikuma (Christian and Adom Farms) and Somanya (Prosper and Boko Farms), which are in the Shai Osudoku and Yilo Krobo Districts of Greater Accra and Eastern regions, respectively. The two localities were selected for the research based on the bimodal climatic conditions for the growing of the crop. Four main criteria were considered before selecting a farm for the research. These are:

1. The three mango cultivars (kent, keitt and palmer) stand to present in the farm
2. Regular spacing between the mango trees (10m x 10m)
3. The mango farmer should be a member of Mango Farmers Association
4. The mango farmer should be willing to give his farm for the research.

The laboratory work was carried out at ARPPIS Laboratory, University of Ghana, Legon.

3.2 Yilo Krobo and Shai Osudoku Districts

Both Yilo Krobo and Shai Osudoku Districts are located in the Coastal Savannah agro-ecological zone of Ghana. The Administrative capital in the Yilo Krobo Districts is Somanya which is one of the twenty-one districts in the Eastern Region. The estimated area of this district is 805sq.km, constituting 4.2 percent of the total area of the Eastern Region. The Yilo Krobo Districts is bounded in the North and East by Lower Manya Krobo District, in the South by Akuapem North District and on the West by New-Juaben Municipal District, East Akim Municipal District and

Fanteakwa District. It lies approximately between latitude 6^{o} 00' and 0^{0} 30North and between longitude 0^{o} 30' and 1^{o} 00' West.

Dodowa is the Administrative capital in the Shai Osudoku District which is one of the ten Districts in the Greater Accra Region. The District shares boundaries with Yilo Krobo and Manya Krobo District and Asuogyaman District to the North respectively, to the East with Ada West, to the West with Akuapim North Municipal and Tema Metropolitan respectively and to the South with Ningo-Prampram District. It is the largest district in the region constituting 41.5 percent of the landmass. The total land area is 1,442 sq.km (144,201), which consists of total cultivable land of 129,600 hectares, and has a coastline stretch of about 37 kms. The Districts is situated in the South-Eastern part of Ghana. It lies approximately between 5^{o} 45 South and 6^{o} 05 North and longitude 0^{o} 05 East and 0^{o} 20 West. The absolute maximum temperature is 40 ^{o}C. Rainfall is generally very low with most of the rains, very erratic in nature and coming mostly between September and November. The mean annual rainfall increases from 762.5ml on the coast to 1220ml to the North and Northeast close to the foothills of Akuapim range and on the summit. The vegetation in these two districts is mainly coastal savannah with small transitional zone along the foothills of the Akuapim - Togo mountain range. The soil type in the two districts is the black clays classified as Akuse series with sandy and sandy-loams in certain areas. The main agricultural activities in these two districts are livestock, crop production and fishing. The crops grown in these two districts are maize, banana, rice, sugarcane, garden eggs, okro, pepper, pawpaw and mango.

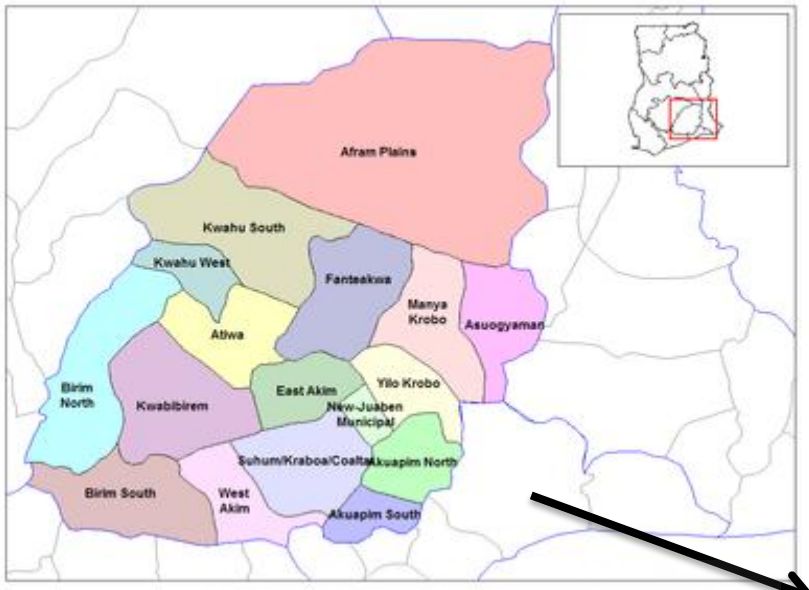


Figure 3.1: Map of the study area (Yilo Krobo and Shai Osudoku Districts)

3.3 Experimental Design

Randomized Complete Block Design (RCBD) with four replications was used in this research. In all, there were a total of twenty plots for the experiment. There were five treatments in each farm. The treatments were as follows:

1. Spent Brewery Grain of Pito (SBGP)
2. Spent Brewery Grain of Brukutu (SBGB)
3. SUCCESS® Appat (GF-120)
4. Great Fruit Fly Bait (GFFB)
5. No Bait spray (as a Control)

Each plot with forty trees was considered for one treatment in each farm. A total number of 200 trees were used for the five treatments in one farm. In all, 800 trees were used for the four different farms. Five plots were randomly allocated at each four different farms. Different coloured ribbons were used to tag the trees to reflect the different treatment. Three rows of plants were used to separate each treatment.

3.4 Treatments

3.4.1 Treatment application method and rates

Four different hand misting spraying equipment were used for the application of treatments, one for each treated plot. These four hand spray machines were boldly marked with a permanent maker to prevent interchanging of equipment during the course of the application of treatments.

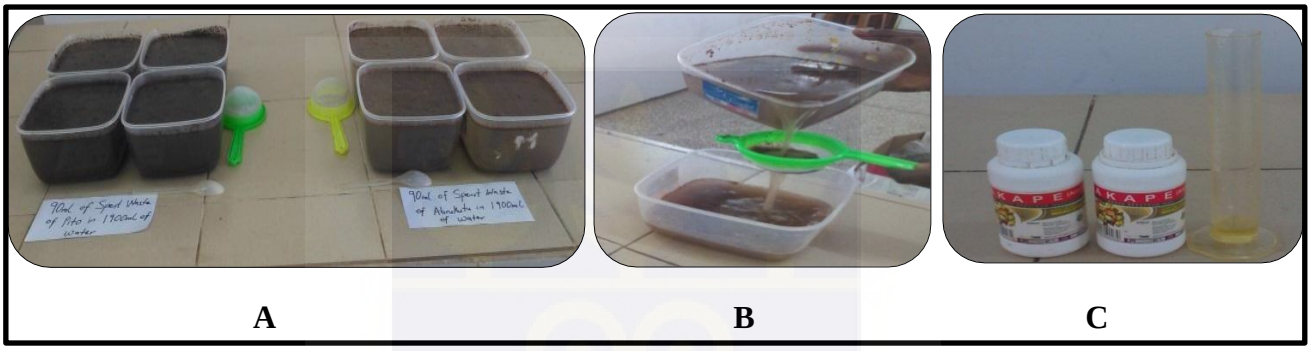
Plate 3.1: Four hand spray equipment

3.4.2 Processing and formulation of Locally-Available Spent Brewery Wastes

Spent wastes of Pito and Brukutu were collected from the local manufacturers. These wastes were sun-dried for a period of two-weeks to eliminate water for good storage. It was milled into fine powder and stored in an air-tight to prevent moulding and contamination from micro-organism.

As a standard measure tried by many countries, including the Africa Fruit Fly Programme (AFFP), 90ml each of the milled spent brewery waste of *Pito* and *Brukutu* was measured in a measuring cylinder. The spent brewery waste of each measured Pito and Brukutu was poured into different square containers containing 1900ml of water and stirred thoroughly for about five minutes. The mixtures in the different square containers were covered with a lid in a room and stored for over 24 hours to allow the protein steep out of the product. The mixtures were then filtered through a very fine net (plate 3.2) to get rid of all debris and the filtrate was poured into another two different square containers. Ten millilitres (10ml) of imidacloprid was added as a killing agent. Imidacloprid is a systemic neonicotinoid produced by the German Chemical Firm

Bayer Crop soil and sold under different trade names such as Gaucho, Admire, Merit, Advantage, Confidor, Provado, and Winner. In this experiment imidacloprid (marketed under the general local names of 'Anty Ataa' and 'Akape®') (Plate 3.2) was used. The chemical acts by interfering with the transmission of stimuli in the insect nervous system. This causes a blockage in the neuronal pathway of the insect. It is a product that is manufactured for soil, seed and foliar application for control of sucking insects. The two different mixtures were then poured into two different hand misting sprayer equipment (Plate 3.1).



A **B** **C**

Plate 3.2:Prepared SBGP and SBGB (A), Straining of Spent Brewery Grain (B), Measured Imidacloprid (Akape®) to be added to the steeped SBGB (C)

3.5 Measuring and mixing of SUCCESS Appat (GF-120)

GF-120 (Active ingredient, Spinosad 0.24 g/L) is a bait that required dilution with water before spraying against the fruit infesting flies. GF-120 (333.3ml) was measured and was poured into a square container containing 2 litres of water. The mixture was stirred vigorously with a stirrer to blend the solution before pouring into different hand misting sprayer equipment to prevent the product sinking and settling to the bottom of the container (Plate 3.1).

3.6 Measuring and mixing of Great Fruit Fly Bait (GFFB)

The Great® Fruit Fly Bait (GFFB) is a bait that requires dilution with water before spraying against the flies. GFFB (333.3ml) was measured and poured in a plastic container containing 1

litre of water. The mixture was vigorously stirred with a rod to blend before pouring into a different hand misting sprayer equipment to prevent the product sinking and settling to the bottom of the container (Plate 3.1).

3.7 Spraying of Treatments

Fifty millilitres (50ml) of coarse droplets was delivered onto one square meter of foliage in such a way that the drift did not hit fruits on the tree. Downward spraying was done to avoid spraying drift to the applicator. Each of the forty trees in a plot was sprayed with the different mixtures of GFFB, GF-120, SBGP and SBGB. Spraying was done early in the morning (6am to 8am) to prevent spray drift. Spraying was repeated weekly on different spot on the foliage to avoid contamination of bait and to eventually increase the treated surface over time in protecting the fruit from infestation by fruit flies. Photo-degradation of the product was prevented by spraying the bait inside the canopy. Applications of treatments were repeated when there was rainfall immediately after spraying.

3.7.1 Control treatment plot

No application of treatments were applied at the control treatment plots in all the four mango farms but traps baited with the different attractant plug with insecticide were deployed in the farms to determine the species composition and the relative fly density.

3.8 Fruit Harvesting and incubation

After 8 weeks of bait applications, infestations of fruit flies were determined by harvesting thirty fruits at random from each plot. This was done in all the four farms. Mature and ripe fruits were selected because they were more likely to be infested than unripe or immature fruits (Mwatawala *et al.*, 2009). The plot name, date and farm name were recorded in a field note book. Fruit samples were placed in individual sacks with the appropriate labels. They were then transported

from the field to the laboratory for incubation. Fruits in each sample were counted and weighed before being placed in incubation containers. The incubation chambers (Plate 3.3) were improvised from two cylindrical semi-transparent plastic containers (28 x 25 x 21cm). One of the cylindrical plastic containers had small holes created at the bottom. The small holes served as a passage for the larvae of the fruit fly to fall to the *soil.*

Plate 3.3: Incubation cylinders

The open end of the cylinder container was covered with a synthetic mesh to allow ventilation. The container with the open end covered with the mesh was immersed into a second container (without holes at the bottom). A layer of moistened sand washed in copious amount of water until all the dust and the dirt particles were removed, was used. The sand was heat-sterilized at 120 ^{o}C for at least 12 hours and cooled at ambient temperature before being used. The lower cylinder had a thin layer of heat-sterilized sand serving as a puparation medium for the larvae that exit from the fruits in the nested container. The cylinders were arranged on a wooden table. The legs of the table were immersed in square container filled with water to help increase humidity and also acting as a barrier to any foreign insect. Each fruit sample was last for a minimum period of 4 weeks at 26 ± 37 ^{o}C and 60 ± 100% RH. Incubated fruits were sprinkled with water as and when necessary to avoid drying and wrinkling. The 4-weeks holding period encompasses the period of larval pupation. Fruits were dissected after incubation to remove any trapped/hidden larvae or

puparia before being discarded as larvae may sometimes prefer to remain inside the rotting fruit if the outside environmental conditions are not conducive (Billah, personal communication).

3.8.1 Insect monitoring

The sand was inspected every three days for the presence of puparia. The puparia were carefully collected with featherweight forceps, counted and placed in petri dishes lined with moistened filter paper. Each petri dish was labeled with the farm name of the experiment, date of collection and number of puparia. Inspection was done until no puparia were present in the sand. As the flies emerged from the puparia in the petri dish, they were released into perspex cages. On opposite sides of the cage were two 10 cm diameter holes. One side sealed with muslin cloth to facilitate ventilation and other a fabric sleeve (Calico) to allow access to the cage by hand. Emerging tephritids were provided with artificial diet of a mixture of yeast powder and brown sugar blended together in a ratio 1: 3, and water in soaked cotton balls (Billah, 2004; Copeland, 2006). The food was blended to make it homogenous and to increase its uptake by the flies. Food and water were served in separate petri dishes and placed inside the cages. Flies were allowed to feed for 3-5 days during which full adult development and body colouration were attained before they were killed in a Killing jar. The dead insects were emptied into plastic vials that had been appropriately labeled and preserved in 70% ethanol.

3.9 Attractants and trap layout for fruit flies

Traps were set up to catch the male fruit flies in the mango farm at the different study sites to determine the species composition and fly population levels. Trapping was done using traps baited with four different attractants (Terpinyl acetate, TA), (Trimedlure, TML), (Cue lure CUE) and (Methyl eugenol, ME). CUE attracts *Zeugodacus* species, TML and TA attract *Ceratitis* species, while ME attract *Bactrocera* species (Ekesi and Billah, 2007). These attractants were

used as solid cylindrical substrates or plugs as this type of formulation makes it possible to have homogeneous doses of the para-pheromones to facilitate comparison of results. One cube of dimethyl 2, 2 – dichlorovinyl phosphate (DDVP) was used as killing agents in the containers per trap. The attractants were released slowly from the polymeric plugs. The solid cylindrical substrates and the killing agent were placed at the bottom in a thin improvised 500ml mineral water bottle trap (5 cm diameter and 20 cm high), with two holes (2×2) cm created on opposite sides of the bottles to serve as the entrance of the insects. Mineral Water Bottle (MWB) fruit fly traps (plate 3.4) were used for the study**.**

A **B** **C** **D**

Plate 3.4: Mineral Water Bottle (MWB) traps with baits and killing agent: Methyl Eugenol (A), Trimedlure (B) Cue lure (C) and Terpinyl acetate (D)

A binding wire was inserted through a central hole in the lid fitted on the trap top, and bent into a hook inside the trap to prevent the wire from slipping through. One hundred and sixty attractant solid cylindrical substrates (Forty (40) of each type), were used to determine the species composition and the relative fly densities. Each plot has two (2) of each trap (that is, 8 per plot). In order to avoid disturbance with each trap, traps were hanged 2.0 - 4.0 m above the ground

(depending on canopy architecture and tree age) and at a distance of 30m from each trap (Ekesi and Billah, 2007).

Traps were hanged in such a manner that leaves and branches were close, but did not touch the traps (to prevent entry of ants and other predators). This was to provide landing and resting places for arriving flies. The different traps (Plate 4.3) were suspended by wire onto branches in a semi-shaded spots in the upward part of the canopy (Ekesi and Billah, 2007). The central part of each wire was smeared with solid grease in order to prevent ants from climbing down the wire to feed on dead adult flies in the traps. All traps were rotated on weekly basis in all the mango farms. Insecticide and para-pheromone cylinders were changed every month.

3.9.1 Fruit fly identification

Traps were emptied at weekly interval into separate labeled vials containing 70% ethanol for preservation. Insect collections were done using a soft camel brush. Once collection was done important information such a location of each trap, name of farm, trap name, date of trap were recorded in a field note book. Collected specimens were sent to ARPPIS Laboratory for identification. Taxonomic identification of both trapped flies and those from incubated fruits was done using literature and keys of White and Elson-Harris (1992), De Meyer (1996, 1998, 2000), Billah and Mansell (2006) and Billah et al. (2007). Identity of some species was further confirmed by Dr. Maxwell K. Billah - a Fruit Fly Taxonomist, Department of Animal Biology and Conservation Science (DABCS), University of Ghana, Legon.

3.9.2 Pre-treatment to determine fruit fly population level and composition

A pre-treatment study was carried out in all the four farms for three consecutive weeks. This was done by collecting and counting the fly catches from traps without any application of treatments. The traps were set up as described in the procedure above. The main aim of the pre-treatment was

to determine the fruit fly population level and species present in the selected farms for the experiment.

3.9.3 Data analysis

Analysis of variance was performed using Genstat Release Version 12 on the total number of different fruit fly species and on the catches of each of the traps in the four different farms. Data were transformed using square root to normalize the variance before analysis. When ANOVA were significant, means were separated using Student Newman Keuls (SNK) test at P = 0.05. Line graphs were drawn using Microsoft Excel to illustrate the relationship between catches for each of the farms and plots.

Calculating infestation level: A) $\frac{\text{Number of puparia}}{\text{Number of fruits}}$ B) $\frac{\text{Number of puparia}}{\text{Weight of fruits (Kg)}}$

The difference in infestation: the infestation level value of control plots - the infestation level value of treated plots (C-T).

The percentage protection: $\frac{\text{Difference in infestation levels between control and treated plot (C-T)}}{\text{Infestation level of the control plot (C)}} \times 100$

CHAPTER FOUR

4.0 RESULTS

4.1 Infestation levels and percentage reduction of incubated fruits

A total of six hundred (600) mango fruits, weighing 400.66 kg were used for the incubation. The number of puparia was highest in the control plots (89) than the other four treated plots. GFFB-treated plots recorded 9 puparia, 24 for SBGB-treated plots, 35 for SBGP treated plots and GF-120plots recorded 38 puparia, as shown in Table 4.5.

Table 4.1 Infestation level of fruits incubated and levels of protection from Prosper Farms

Treatment	Number of Fruits	Weight of Fruits (Kg)	Number of puparia	Infestation level		Difference (C-T)	% Protection $(\frac{C-T}{C})\times 100$
				Puparia/Fruit	Puparia/Weight (kg)		
SBGP	30	18.79	8	0.267	0.426	0.665	60.95
SBGB	30	22.07	7	0.233	0.317	0.774	70.94
GF-120	30	20.12	11	0.367	0.547	0.544	49.86
GFFB	30	20.47	4	0.133	0.195	0.896	82.13
Control	30	20.17	22	0.733	1.091		

Table 4.2 Infestation rate of fruits incubated and levels of protection from Boko Farms

Treatment	Number of Fruits	Weight of Fruits (Kg)	Number of puparia	Infestation level		Difference (C-T)	% Protection $(\frac{C-T}{C})\times 100$
				Puparia/Fruit	Puparia/Weight (kg)		
SBGP	30	19.00	5	0.167	0.263	0.744	73.88
SBGB	30	20.28	4	0.133	0.197	0.810	80.44
GF-120	30	19.08	8	0.267	0.419	0.588	58.39
GFFB	30	21.87	1	0.033	0.046	0.961	95.43
Control	30	19.87	20	0.667	1.007		

Table 4.3 Infestation level of fruits incubated and levels of protection from Christian Farms

Treatment	Number of Fruits	Weight of Fruits (Kg)	Number of puparia	Infestation level		Difference (C-T)	% Protection $(\frac{C-T}{C})\times 100$
				Puparia/Fruit	Puparia/Weight (kg)		
SBGP	30	18.84	12	0.400	0.637	0.662	50.96
SBGB	30	22.34	8	0.267	0.358	0.941	72.44
GF-120	30	18.87	10	0.333	0.530	0.769	59.20
GFFB	30	18.10	2	0.067	0.110	1.189	91.53
Control	30	18.47	24	0.800	1.299		

Table 4.4 Infestation level of fruits incubated and levels of protection from Adom Farms

Treatment	Number of Fruits	Weight of Fruits (Kg)	Number of puparia	Infestation level		Difference (C-T)	% Protection $(\frac{C-T}{C})\times 100$
				Puparia/Fruit	Puparia/Weight (kg)		
SBGP	30	19.73	10	0.333	0.507	0.587	53.66
SBGB	30	23.47	5	0.167	0.213	0.881	80.53
GF-120	30	19.09	9	0.300	0.471	0.623	56.95
GFFB	30	19.00	2	0.067	0.105	0.989	90.40
Control	30	21.03	23	0.767	1.094		

Ranges of Fruit Protection Levels:

1. SBGP = 50.96 - 73.88
2. SBGB = 70.94 - 80.44
3. GF-120 = 49.86 - 59.20
4. GFFB = 82.13 - 95.43

Table 4.5. Summaries of the infestation level of fruits incubated in all selected farms

Treatments	Total number of Fruits	Total weight of Fruits (Kg)	Total number of puparia	Infestation level		Difference (C-T)	% Protection $(\frac{C-T}{C})\times 100$
				Puparia/Fruit	Puparia/Weight (kg)		
SBGP	120	76.36	35	0.292	0.458	0.661	59.07
SBGB	120	88.16	24	0.200	0.272	0.847	75.69
GF-120	120	77.16	38	0.317	0.492	0.627	56.03
GFFB	120	79.44	9	0.075	0.113	1.006	89.90
Control	120	79.54	89	0.742	1.119		

4.2 Flies from incubated fruits

A total of one hundred and ten (110) fruit flies were reared from the sampled mango fruits for the study. Only *Bactrocera dorsalis* species of fruit flies emerged from all the incubated fruits. No parasitoids were reared. Control treatment plots recorded the highest number of fruit flies, and the least was the GFFB-treatment plots. The number of fruit flies that emerged from the different treatments are summarized in Table 4.6.

Table 4.6. Fruit flies emerging from sampled mangoes in the different treatments

Treatment	Tephritid species	Number of flies	% of emerged flies
SBGP	*Bactrocera dorsalis*	9	8.18
SBGB	*Bactrocera dorsalis*	6	5.45
GF-120	*Bactrocera dorsalis*	12	10.90
GFFB	*Bactrocera dorsalis*	4	3.64
Control	*Bactrocera dorsalis*	79	71.81
Total		110	

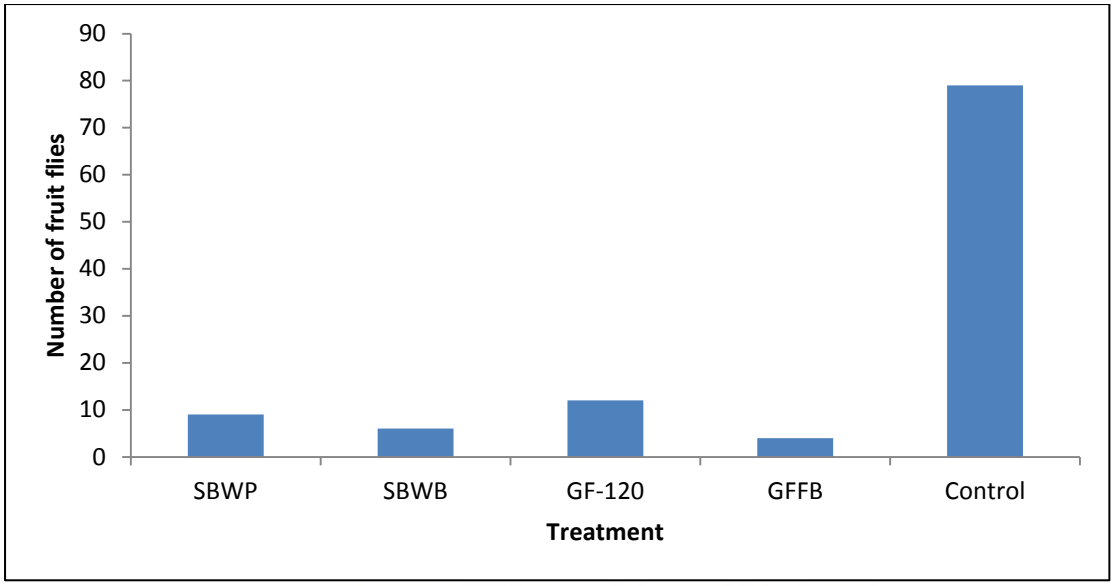


Figure 4.1: A graph showing the number of fruit fly that emerged after incubation

4.3 Composition of fruit flies species in mango farms

A total of 155,170 insects were collected during the study, of which 152,565 (98.32%) were fruit flies and 2,605 (1.68%) as non-target organisms. During the three weeks of pre-treatment to determine the presence of fruit flies in the four different farms, a total of 9,106 insect were collected. Out of this 8,890 (97.63%) were fruit flies and 216 (2.37%) as non-target. A total of 146,064 insects were collected during the treatment period, of which 143,675 (98.36%) were collected as fruit flies and 2,389 (1.64%) were non-target. Three genera containing four different species of fruit flies were recorded. These include *Bactrocera dorsalis, Zeugodacus cucurbitae, Ceratitis capitata* and *Ceratitis cosyra.*

4.4 Trap catches of fruit flies at Prosper Farms

Catches of fruit flies by Methyl eugenol traps during the pre-treatment recorded 95 flies in week 1, and attained the highest in week 3 (366). During the treatment period, Methyl eugenol traps recorded the highest catches in week 6 (3,746) and the least in week 1 (1,693). No fruit fly catches were recorded during the pre-treatment in TA traps. Four (4) fruit flies were recorded in TA traps in week 4, and did not collect again till week 11 during the treatment period. No fruit flies were collected in the TML traps during the pre-treatment period, while six (6) fruit flies were recorded in week 6. No more flies were collected till week 11 during the treatment period. No catches were made in week 1 during the pre-treatment, but in week 3, thirteen (13) fruit flies were recorded in the CUE traps. The highest catches of fruit flies in CUE traps were recorded in week two (21) and the least in week seven (4) during the treatment period. As showed in Fig. 4.2.

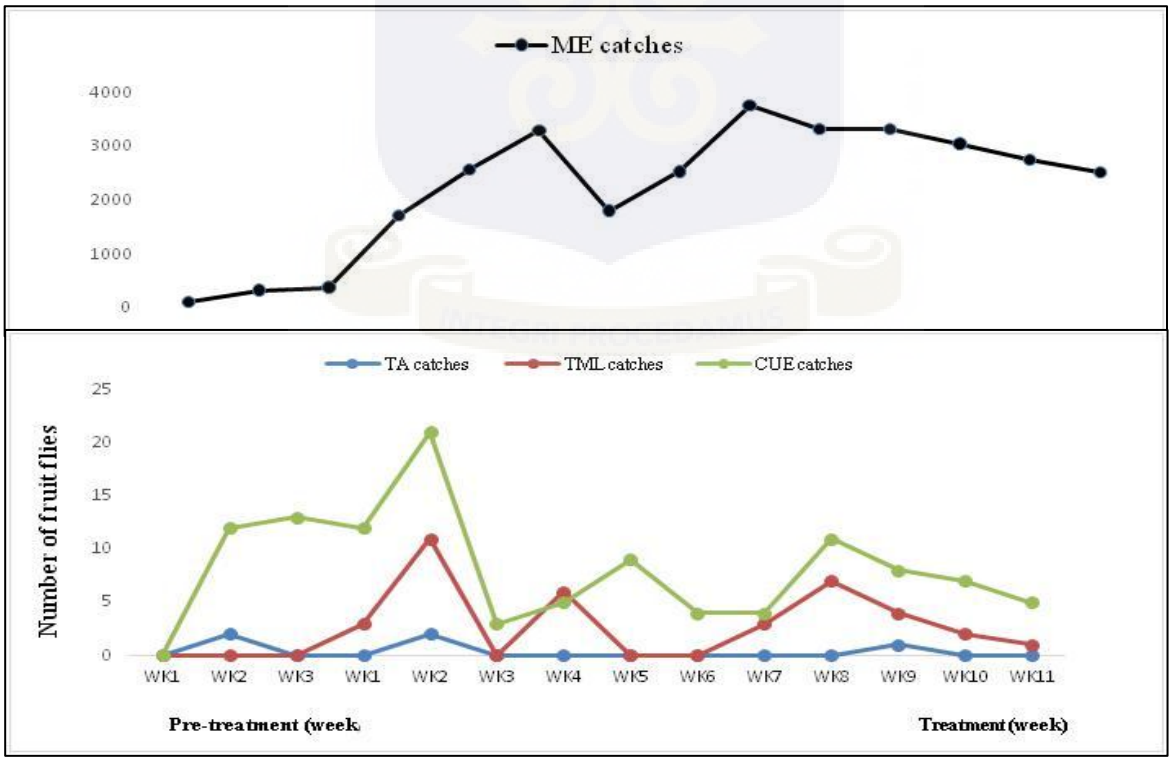


Figure 4.2: A graph showing catches of fruit fly with the different baited traps at Prosper farm

4.5 Trap catches of fruit flies at Boko Farms

Methyl Eugenol (ME) traps recorded one hundred and twenty-five (125) fruit flies in pre-treatment week 1, and recorded the highest in week 3 (531). The highest catches were recorded in week 7 (1,286), and the least in week 11 (924) in ME traps during the treatment period. No fruit fly was recorded in weeks 1 and 2 (2) during the pre-treatment period in TA traps, but three (3) fruit flies were recorded in week 3. No catches were recorded in TA traps from weeks 1-7, where four (4) fruit flies were recorded and the number remained zero till week 11. For TML traps, eleven (11) fruit flies were recorded in the third week during the pre-treatment period. During the treatment period, the highest were recorded in week 5 (20), and the least in week 11 (4). CUE traps recorded thirty-three (33) fruit flies in the third week as the highest during the pre-treatment period. CUE traps collected thirty two (32) fruit flies in week 2, and the least were recorded in weeks 10 and 11 (11) during the treatment period (Fig.4.3).

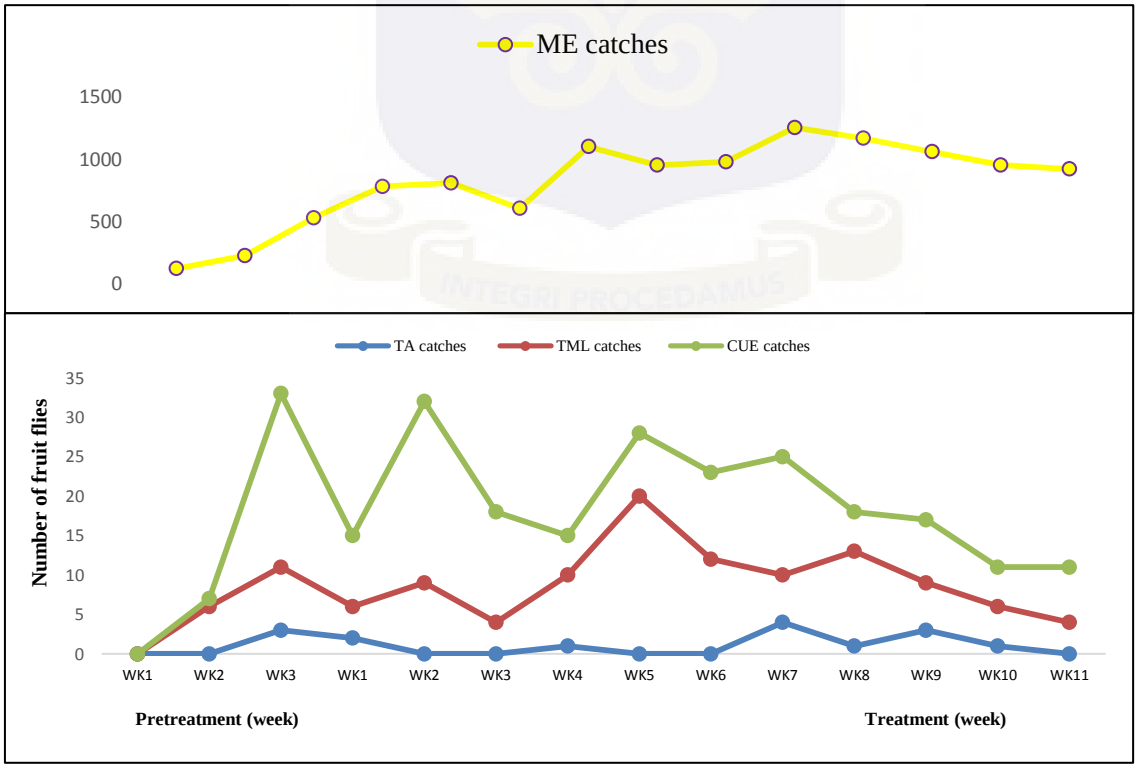


Figure 4.3: A graph showing catches of fruit fly with the different baited traps at Boko Farms

4.6 Trap catches of fruit flies at Christian Farms

ME traps recorded 1,058 fruit flies during pre-treatment week 1 and the highest in week 3 (2,301). The highest catches of fruit flies was recorded in week 7 (7,180), and the least number by ME traps was recorded in week 2 (4,246). TA traps recorded no flies during the pre-treatment period, and (11) fruit flies were recorded as the highest in week 8 whilst no catches were recorded in week one during the treatment period. TML traps recorded no flies in weeks 1 and 2, but recorded 5 flies in week 3 during the pre-treatment period. During the treatment period, twelve (12) fruit flies were recorded in week 4 as the highest and the least in week 1 TML traps. No fruit fly was recorded by CUE traps in week 1, but 24 flies were recorded in week 3 during the pre-treatment period. The highest catches of 29 fruit flies by CUE traps were recorded in week 4, and the least number of 11 flies recorded in weeks 10 and 11 (Fig. 4.4).

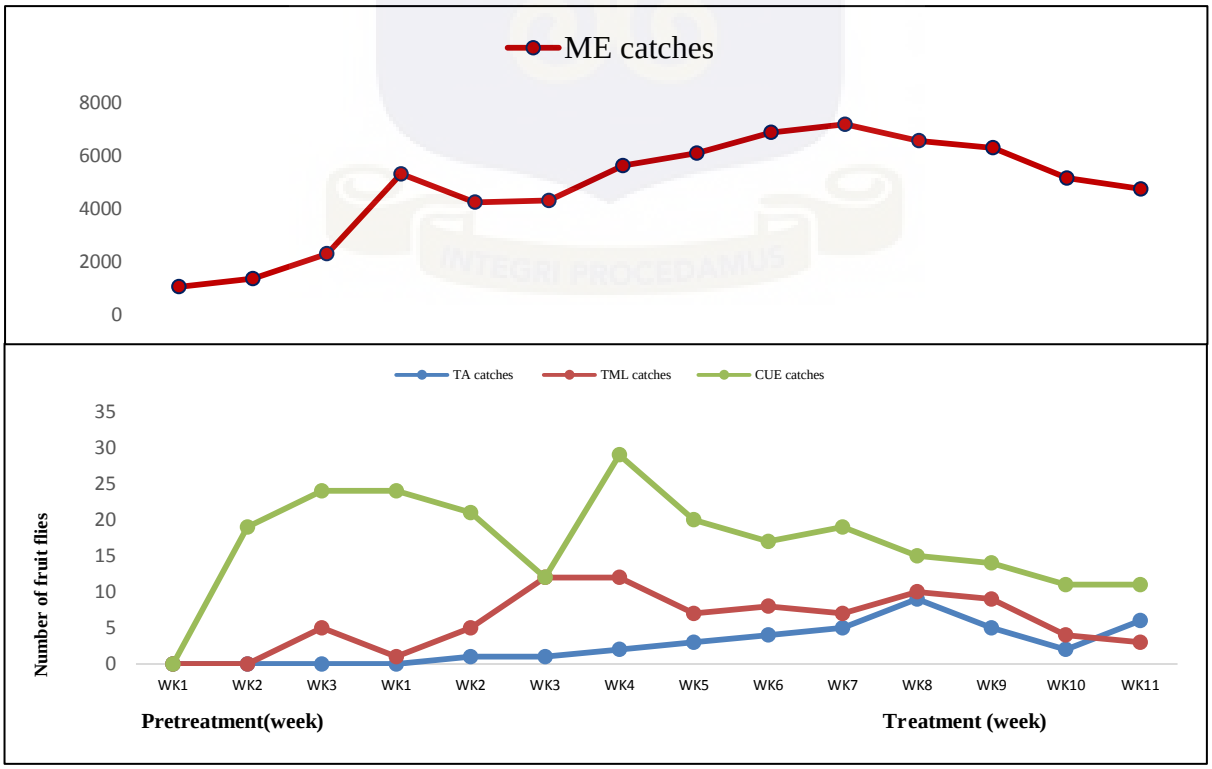


Figure 4.4: A graph showing catches of fruit fly with the different baited traps at Christian farm

4.7 Trap catches of fruit flies at Adom Farms

During the pre-treatment period, ME traps recorded 1,371 fruit flies in week 3 as the highest, and the least of 219 recorded in week 1. Four thousand, two hundred and sixty two (4,262) fruit flies were recorded as the highest in week 4,while 2,160 were recorded in week 1 during the treatment period ME traps. Two (2) fruit flies were recorded as the highest catches in week 3 during pre-treatment by TA traps. During the treatment period, five (5) fruit flies were recorded in week 5, while 10 catches of fruit flies were made in week nine and ten by the TA traps. Two (2) fruit flies were trapped by the TML traps in week 2 during the pre-treatment. Week 3 recorded the highest catches of 34 fruit flies by TML traps, and the least number of 5 recorded in week 9 during the treatment period. Twenty (20) fruit flies were recorded as the highest catches in week 3, with no catches made in week 1 during the pre-treatment period by CUE traps. Twenty nine (29) fruit flies were recorded in week 6 and the least number of 11 recorded in week 11 by CUE traps during the treatment period (Fig. 4.5).

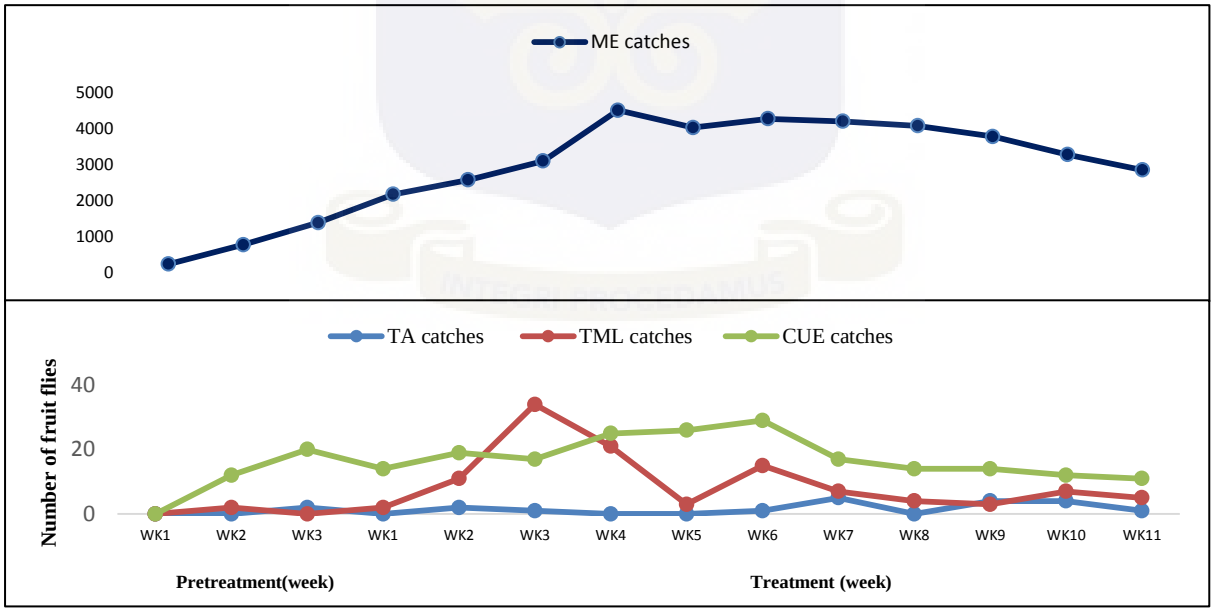


Figure 4.5: A graph showing catches of fruit fly in the different baited traps at Adom farm

Table 4.7 Performance of different lures in terms of fly catches in four localities

Lures	Statistical Parameters	Trap Catches		
		Pre-Treatment Period	Treatment Period	Total Catches
		Prosper Farms		
ME		15.503 ± 2.9b	52.27 ± 1.912b	44.39 ± 4.472b
TA		0.9985 ± 0.2913a	0.834 ± 0.088a	0.869 ± 0.089a
TML		0.7071 ± 0a	1.77 ± 0.27a	1.542 ± 0.24a
CUE		2.639 ± 0.967a	2.83 ± 0.24a	2.79 ± 0.26a
	F	21.25	671.65	90.54
	Df	11	43	55
	P	<.001	<.001	<.001
		Boko Farms		
ME		16.443 ± 3.50b	30.93 ± 0.92c	27.82 ± 1.91b
TA		1.095 ± 0.39a	1.162 ± 0.15a	1.148 ± 0.14a
TML		2.216 ± 0.80a	3.066 ± 0.22b	2.884 ± 0.24a
CUE		3.078 ± 1.48a	4.4 ± 0.23b	4.12 ± 0.36a
	F	13.66	814.24	165.74
	Df	11	43	55
	P	0.002	<.001	<.001
		Christian Farms		
ME		39.14 ± 4.596b	75.04 ± 2.04b	67.35 ± 4.46b
TA		0.7071 ± 0a	1.876 ± 0.21a	1.625 ± 0.21a
TML		1.2531 ± 0.55a	2.668 ± 0.22a	2.365 ± 0.25a
CUE		3.36 ± 1.33a	4.2 ± 0.20a	4.02 ± 0.30a
	F	60.43	1213.76	208.33
	Df	11	43	55
	P	<.001	<.001	<.001

		Adom Farms		
ME		26.45 ± 6.44b	58.99 ± 2.05b	52.01 ± 4.20b
TA		0.9985 ± 0.29a	1.334 ± 0.19a	1.262 ± 0.16a
TML		0.9985 ± 0.29a	3.01 ± 0.40a	2.579 ± 0.39a
CUE		2.92 ± 1.15a	4.25 ± 0.21a	3.97 ± 0.30a
	F	14.43	710.9	136.81
	Df	11	43	55
	P	0.001	<.001	<.001

*Mean in same column followed by different letters are statistically different at (p=0.05), using Student-Newman-Keuls (SNK) test. ANOVA performed using square root transformed proportion values.

Analysis of Variance (ANOVA) of the pre-treatment, treatment and total catches was statistically significant in all the study farms (Prosper, Boko, Christian and Adom Farms). Student-Newman-Keuls' post hoc test showed that ME lure was significantly higher than the other lures (TA, TML, CUE) in all the study farms in both pre-treatment and total catches. The other lures catch however were not statistically significant from each other Table 4.7.

During treatment period, all the lures in Boko Farms were significantly different in catches except for TML and CUE lures which were not different statistically. However, ME lure was significantly higher than TA, TML and CUE lures in the other study farms (Prosper, Christian and Adom Farms).

4.8 Fruit fly captures

The relative population density of flies for the pre-treatment and treatment periods were recorded as an "average daily trap catches". Thus, the number of specimens collected in a location was divided by number of traps set in each plot and by the number of days of trap exposure (IAEA, 2003: Ekesi and Billah, 2006). The function of this index is to have a relative measure of the size of adult population in a given space and time. This is a standard

index that allows comparison of trap catches across different localities without regard to local conditions. According to International Atomic Energy Agency (IAEA) (2003), fruit fly density is the ratio of the number of flies per trap per day, that is;

$$\text{FTD} = \frac{\text{Total number of flies trapped}}{\text{Total number of serviced traps} \times \text{number of days traps exposed in the field}}$$

The trap data collected during the pretreatment and treatments periods were used to estimate the relative fly density for the various locations (Table 4.8, 4.9).

Table 4.8 Relative fly density of fruit fly in Prosper and Boko farm

Locality/Plot	Fruit fly species	Pretreatment period				Treatment period			
		No. of flies	No. of traps	Exposure Period (days)	Flies/ Trap/ Day	No. of flies	No. of traps	Exposure Period (days)	Flies/ Trap/ Day
Prosper Farms	*Bactrocera dorsalis* (ME)	770	10	21	3.667	30,856	10	79	39.058
	Zeugodacus cucurbitae (CUE)	25	10	21	0.119	89	10	79	0.l13
	Ceratitis capitata (TML)	0	10	21	0	32	10	79	0.041
	Ceratitis Cosyra (TA)	2	10	21	0.010	3	10	79	0.004
Boko Farms	*Bactrocera dorsalis* (ME)	883	10	21	4.205	10,610	10	79	13.430
	Zeugodacus cucurbitae (CUE)	40	10	21	0.190	213	10	79	0.2696
	Ceratitis capitata (TML)	15	10	21	0.071	104	10	79	0.136
	Ceratitis Cosyra (TA)	3	10	21	0.014	12	10	79	0.015

Table 4.9 Relative fly density of fruit fly in Christian and Adom

Locality/Plot	Fruit fly species	Pretreatment period				Treatment period			
		No. of flies	No. of traps	Exposure Period (days)	Flies/ Trap/ Day	No. of flies	No. of traps	Exposure Period (days)	Flies/ Trap/ Day
Christian farm	*Bactrocera dorsalis* (ME)	4,722	10	21	22.486	62,387	10	79	78.971
	Zeugodacus cucurbitae (CUE)	45	10	21	0.214	193	10	79	0.244
	Ceratitis capitata (TML)	5	10	21	0.024	79	10	79	0.1
	Ceratitis Cosyra (TA)	0	10	21	0	41	10	79	0.052
Adom farm	*Bactrocera dorsalis* (ME)	2,346	10	21	11.17	38,728	10	79	49.022
	Zeugodacus cucurbitae (CUE)	30	10	21	0.143	198	10	79	0.251
	Ceratitis capitata (TML)	2	10	21	0.009	12	10	79	0.015
	Ceratitis Cosyra (TA)	2	10	21	0.009	18	10	79	0.022

4.9 Non- target captures

A total of 2,605 non-target insect species were collected during the study. 216 (8.29%) were collected during the pretreatment period and the rest 2,389 (91.71%) collected during the treatment period (Table 4.10). During the pretreatment, GF-120 plots (Table 4.13)recorded the highest number of non-target insect species 62 representing 28.70% followed by SBGB plots, SBGP plots, Control plots and GFFB plots recording 55 (25.46%), 41 (18.98%), 30 (13.89%) and 28 (12.96%) species respectively in that order. The highest insect Order collected during the pretreatment was from the Order Araneae (Table 4.12) and the least from the Order Odonata (Table 4.11). Methyl eugenol traps recorded the highest catches of non-target and the least was from the Terpinyl acetate. During the treatment period GF-120 plots (Table 4.13) recorded the highest number of non-target species as 736 (30.81%) followed by SBGB plots, GFFB plots, SBGP plots and Control plots constituting 552 (23.11%), 390 (16.32), 379 (15.86%) and 332 (13.90) respectively in decreasing order of catches. GF-120 plot at Prosper farm recorded the highest number of catches of non-target as 214 and the least was also recorded in the Christian farm at control plot (29) during the treatment period (Table 4.15). The highest insect Order trapped during the treatment period was from the Hymenoptera (Table 4.13) and the least was from the Order Odonata (Table 4.14). Methyl eugenol trapped the highest number of non-target followed by Cue lure, Trimedlure and Terpinyl acetate during the treatment period. Range of non-target insect caught by the different attractants during the study were Spider (Araneae), beetles (Coleoptera), ants (Hymenoptera), tree cockroaches (Blattodea), moths (Lepidoptera), flies (Diptera), dragonflies (Odonata) and grasshoppers (Orthoptera).

Table 4.10 Number of non-target species captured during the study

Treatment	Pre-treatment	Application of Treatment
SBGP	41	379
SBGB	55	552
GF-120	62	736
GFFB	28	390
CONTROL	30	332
TOTAL	216	2,389
	2,605	

Treatments: SBGP – Spent Brewery Grain of *Pito*

SBGB – Spent brewery Grain of *Brukutu*

GF-120 – SUCCESS Appat

GFFB – Great Fruit Fly Bait

Table 4.11 Number of non-target species captured at SBGP plots in the four farms

Non-target species	**SBGP**							
	Pretreatment				**Treatment**			
	PR	**BO**	**CH**	**AD**	**PR**	**BO**	**CH**	**AD**
Coleoptera	3	0	2	0	34	14	21	12
Diptera	1	0	4	0	6	45	43	12
Hymenoptera	2	7	3	0	3	34	25	14
Lepidoptera	0	2	0	0	2	0	1	2
Araneae	2	10	0	4	9	32	43	3
Blattodea	0	0	0	0	7	1	1	2
Orthoptera	0	0	0	1	3	2	2	5
Odonata	0	0	0	0	0	0	0	1
TOTAL	8	19	9	5	64	128	136	51
	41				**379**			

Localities: PR=Prosper farm BO=Boko farm CH=Christian farm AD=Adom farm

Table 4.12 Number of non-target species captured at SBGB plots in the four farms

Non-target species	SBGB							
	Pretreatment				Treatment			
	PR	BO	CH	AD	PR	BO	CH	AD
Coleoptera	2	4	3	2	12	23	47	23
Diptera	2	0	0	0	34	45	23	12
Hymenoptera	0	0	0	0	56	25	12	45
Lepidoptera	0	2	0	1	4	23	13	32
Araneae	0	23	0	3	34	24	34	12
Blattodea	0	2	0	2	2	2	2	2
Orthoptera	0	0	1	2	1	0	4	0
Odonata	0	0	2	4	4	0	2	0
TOTAL	4	31	6	14	147	142	137	126
	55				**552**			

Localities: PR=Prosper Farms BO=Boko Farms CH=Christian Farms AD=Adom Farms

Table 4.13 Number of non-target species captured at GF-120 plots four farms

Non-target species	GF-120							
	Pretreatment				Treatment			
	PR	BO	CH	AD	PR	BO	CH	AD
Coleoptera	4	2	4	3	23	34	35	45
Diptera	8	2	0	6	38	23	23	24
Hymenoptera	0	15	0	0	67	45	70	25
Lepidoptera	0	1	0	2	54	12	23	67
Araneae	0	1	0	2	4	34	34	12
Blattodea	3	1	0	2	3	0	2	12
Orthoptera	0	0	0	1	23	0	1	1
Odonata	0	2	0	3	2	0	0	0
TOTAL	**15**	**24**	**4**	**19**	**214**	**148**	**188**	**186**
	62				**736**			

Localities: PR=Prosper farm BO=Boko farm CH=Christian farm AD=Adom farm

Table 4.14 Number of non-target species captured at GFFB plots in the four farms

Non-target species	GFFB							
	Pretreatment				Treatment			
	PR	BO	CH	AD	PR	BO	CH	AD
Coleoptera	2	0	2	1	12	45	34	34
Diptera	1	0	0	0	23	5	6	45
Hymenoptera	3	0	0	1	32	45	23	0
Lepidoptera	0	0	0	0	3	0	2	0
Araneae	5	0	0	0	27	0	45	0
Blattodea	0	2	4	0	0	0	0	5
Orthoptera	0	2	2	1	0	0	0	4
Odonata	0	0	0	2	0	0	0	0
TOTAL	**11**	**4**	**8**	**5**	**97**	**95**	**110**	**88**
	28				**390**			

Localities: PR=Prosper farm BO=Boko farm CH=Christian farm AD=Adom farm

Table 4.15 Number of non-target species captured at Control plots in the four farms

Non-target species	CONTROL							
	Pretreatment				Treatment			
	PR	BO	CH	AD	PR	BO	CH	AD
Coleoptera	1	0	2	3	23	34	2	44
Diptera	0	0	3	3	56	3	3	23
Hymenoptera	0	0	0	0	4	45	2	5
Lepidoptera	0	0	0	0	3	9	7	7
Araneae	0	1	0	0	4	0	8	23
Blattodea	0	2	1	0	4	0	7	0
Orthoptera	1	4	3	0	7	0	0	0
Odonata	2	1	2	1	9	0	0	0
TOTAL	**4**	**8**	**11**	**7**	**110**	**91**	**29**	**102**
	30				**332**			

Localities: PR=Prosper farm BO=Boko farm CH=Christian farm AD=Adom farm

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Efficacy of Spent Brewery Grain of *Pito* and *Brukutu* in the management of fruit flies

Spent brewery grains used for controlling fruit flies have been tried in other regions of the world, for example Australia (Lloyd and Drew, 1997), Mauritius (Sookar *et al*., 2002) and China (Zhou *et al.*, 2012) Ghana (Banini 2013) on Mango and (Bulley 2012) on Citrus. After the incubation to determine infestation levels, only *Bactrocera dorsalis* emerged, out of the four (4) tephritid fruit fly species trapped. *Zeugodacus cucurbitae*, *Ceratitis cosyra* and *Ceratitis capitata* were detected by the traps but there were no record of infestation on mangoes at the study sites. The number of *Bactrocera dorsalis* emerging from the mangoes incubated suggested the dorminace of this species at the onset of fruiting in the mango farm. This confirms the work done by Ekesi *et al*. (2009) indicating that *Bactrocera dorsalis* have displaced indigenous fruit fly and become the most abundant fruit fly pest of mango**.** The percentage protection of SBGP and SBGB are 57.07 and 75.69, respectively. These results further indicate that the relative amount of bait applied per tree on the leaves has a significant effect on reducing fruit infestation levels. This confirms the work done by Chinajariyawong *et al*. (2003) when they evaluated the Australian protein bait, Pinnacle® (Mauri Yeast, Camellia, NSW, Australia) and brewery waste from Thailand, and found that both baits significantly reduced fruit fly infestation when compared with controls. Also in Malaysia, a yeast autolysate, Promar, was used successfully to control large populations of fruit flies in Carambola Plantations (Australian Centre for International Agricultural Research, 2014). It was proven to be an excellent attractant for local species of fruit flies and does not cause phytotoxicity to plant (Vijaysegaran, 1989; Loke *et al*., 1992). The results also confirms field experiment conducted in Fiji using protein bait sprays indicating that these locally produced

protein baits could be used to effectively control fruit flies in both commercial and wild stands of guava (Australian Centre for International Agricultural Research, 2014). However, comparing the two Spent Grain of Brukutu and Pito, Spent Grain of Brukutu offers more protection to the mango fruits than Spent Grain of Pito. According to Robertson *et al*. (2010) the variation in percentage composition of the components in any spent grain is attributable to the variety of the grains used, harvest time, malting and mashing conditions, and the quality and type of adjuncts used during the process. From this result, plots sprayed with Spent Grain of Brukutu had more protein which were able to attract the insect and kill them on consumption before ovipositing and this resulted in higher protection than Spent Grain of Pito.

The widely used control method in fruit flies suppression is insecticide sprays, due to the ease of accessibility and cost, but, the broad spectrum application of insecticides poses issues of environmental concern such as, environmental pollution and adverse effects on non-targets (Kumar *et al*., 2011). In addition to the above, the cover spraying mode of application (spraying whole plants) requires frequent applications of chemicals which leave chemical residue in the produce and also grains chemicals (Secretariat of the Pacific Community, 2002). Over the past ten years, fruit fly suppression has been based on the use of protein bait mixed with a killing agent that attract and kill fruit flies on consumption of the product due to the specificity and safe mode of action (Vargas *et al*., 2005).The small amount of killing agent added to poison the protein makes its environmentally safe. Poisoned protein bait that are sprayed on spot parts of tree such as stems or leaves prevents grain of insecticide and leaves no chemical residue on fruits since the baits are not directly applied to fruits (Secretariat of the Pacific Community, 2002). IAEA (2007) and Heath *et al*. (2009) recognized the need to develop improved lures and "attract-and-kill" devices for successful

fruit fly control. In view of this, successful manufacturing of protein bait from locally Spent Brewery Grain of Pito and Brukutu with killing agent could be used as alternate and subsequent improved source of cheaper protein bait for fruit flies management.

5.2 Comparing the performance of locally Spent Brewery Grain (Pito and Brukutu) and the Standard products (GFFB and SUCCESS APPAT)

Presently, the intensive use of insecticides is being discouraged as a result of their negative impact on human health and the environment. Environmentally safe pesticides and appropriate protein sources (Peck and McQuate, 2000) are used as baits, as spot spray or in traps to reduce fruit fly populations below the economic threshold.Lloyd and Drew(1997) reported that grain brewery yeast is a rich source of the protein (providing all essential amino acids), B-complex vitamins, and minerals which are needed for insect growth and fecundity. Research has indicated that Spent Brewery Grain mixed with a killing agent is used to suppress fruit fly. An imidachloprid, systemic insecticide was added to the Locally Spent Brewery Grain and it was effective in reducing the fruit flies infestation.

Infestations were recorded in the five plots in all the four farms, as female flies at all stages of sexual maturity prefer laying eggs in fruits than looking for food. This confirmed the work done by Sabine (1992) that gravid female Queensland fruit flies are more interested in laying of eggs in fruits than searching for food. Treatments were applied after fruit set started, so some fruits were already infested by fruit flies; to be effective, treatments should have started 5–10 weeks earlier (before blossom) to control the initial fruit fly population. The fruits in the control plots were not protected especially from the female flies that were searching for suitable hosts for oviposition and this resulted in high infestation level of 1.119 than the other treated plots. After the incubation, the percentage protection for Great Fruit Fly Baits (GFFB)

was 89.90, 75.69 for Spent Brewery Grain of Brukutu (SBGB), 57.07 for Spent Brewery Grain of Pito (SBGP) and 56.03 for SUCCESS Appat (GF-120). GFFB was significantly superior to SBGB, which was superior to the SBGP which was in turn superior to GF-120. From the result obtained GFFB had a higher chance of protecting the crop than the other four treatments. This confirms the work done by Ecoman Biotech in Ghana when they carried out research on the*Bactrocera dorsalis* (Hendel) which was causing more than 50% overall damage rate to the fruits. After the GFFB protein bait application they concluded that, there was significant reduction in fruit damage rate caused by the fruit fly from 30% - 40% to less than 1%, when compared with the same season of the previous year (Ecoman, 2015).This also confirms the work done in Samut Sakhon Province, Thailand when they performed trails in plots of several crops like – mango,rose apple,guava,grape and lemonwhich were mostly infested by*Bactrocera dorsalis* (Hendel). They indicated that average fruit damage rate in treated plots was 1.96 percent while in untreated was 38 percent. The fruit flies damage rate was significantly reduced in plots treated with GFFB (Ecoman, 2015).

From the result obtained locally Spent Brewery Grain of Pito and Brukutu performed better than the GF-120 even in its crude form. This also confirms the work done by Zhou *et al*.(2012) when they evaluated new bait containing an enzymatically hydrolyzed protein produced by the industrial processing of beer yeast, feeding stimulants, orange juice and brown sugar. They concluded that this new bait, termed H-protein bait, outperformed GF-120 in citrus orchards in China. This was also in agreement with the work done in 2007, when Smith and Gutierrez (2008) evaluated the use of GF-120 Bait for the control of Cherry Fruit Fly. The full application of GF-120 greatly reduced but did not completely control Cherry Fruit Fly infestation on sites with high numbers of adults emerging during the first season of treatments. This also confirms work done by Banini (2013) by evaluation of the Grain

Brewers' yeast and GF-120 against fruit flies in mango farms. He concluded that GF-120 and Grain Brewer' yeast offer percentage protection of 33% and 46% respectively in the fields used. Also when the GF-120 were sprayed on a single tree to suppress the larval populations, the bait was not able to eliminate fruit flies after one season, because some flies were not attracted to and did not feed on the GF-120 (Yee and Chapman, 2005;Yee, 2006), or some flies oviposited before bait ingesting and dying. However, GF-120 Naturalyte ™ is only efficient against fruit flies feeding (Prokopy *et al*., 2005), and only if used continuously throughout the time of fruiting and post-fruiting seasons (Revis *et al*., 2004). In Benin during 2006 and 2007 a weekly treatment of GF-120 on mango farms resulted in 80% reduction of damage caused by fruit flies compared to the control farms (Vayssières*et al.*, 2009b).

5.3 Composition of fruit flies species

Monitoring, detection and control of tephritid fruit flies often rely on the deployment of attract-and-kill devices baited with male-specific attractants, termed male lures (Vargas *et al*., 2010, 2014). Monitoring of fruit flies using four different attractants in the four different farms observed four (4) different species. These included *Bactrocera dorsalis, Zeugodacus cucurbitae, Ceratitis cosyra* and *Ceratitis capitata.* They belong to three fruit fly genera (*Bactrocera, Zeugodacus* and *Ceratitis*) which have been reported to be of economic importance in equatorial Africa (White and Elson-Harris, 1992; Ekesi and Billah, 2006). *Bactrocera dorsalis* catches were greater than other species across the four different farms, suggesting the domination of *Bactrocera dorsalis* over the native *Ceratitis* species since its introduction to Africa in 2003, and Ghana in 2005. Also, according to Roomi *et al*.(1993) methyl eugenol has both phagostimulatory as well as olfactory action and can lure fruit flies from a range of 800meters, indicating its effectiveness in managementof *Bactrocera dorsalis.Zeugodacus cucurbitae, Ceratitis capitata* and *Ceratitis cosyra* were the second,

third and fourth in ranking respectively after *Bactrocera dorsalis*, but the numbers of those were significantly lower across the four different farms. Lux *et al*. (2003b) and Afreh-Nuamah (2007) reported that *C. capitata* has been the major pests of citrus in Africa prior to the detection of *Bactrocera dorsalis*. Lux *et al*. (2003b) stated that mangoes were less attacked by *C. capitata.* This suggests that under normal conditions, this pest will prefer other host plants and will only use mango as a host under conditions where their preferred host is not available. The relatively low catches of *Ceratitis cosyra* in the traps suggest that this species migrate out of mango fields unto other susceptible host such as the vegetable farms (example pepper, garden eggs etc) and wild fruiting trees nearby when mango availability is decreasing but return to the farm when fruits were getting matured. This is because *Ceratitis cosyra* are competitively inferior to *B. dorsalis* (Ekesi *et al*., 2009). It is therefore possible that when the population of *Bactrocera dorsalis* start building up within the orchard, that of *Ceratitis cosyra* becomes rapidly displaced due to the inability of the former to use the available food resources to its advantage for its rapid development. *Bactrocera dorsalis* was reported to have displaced *Ceratitis cosyra* and became the predominant fruit fly pest in mango(Ekesi *et al.*, 2009). The presence of this pest in the traps might have resulted from being attracted by the Trimedlure traps from neighbouring pepper and eggplant farms.

The capture rates for *Bactrocera dorsalis* from the start of the study were high during the treatments application. This situation could be attributed to migration of flies from adjacent fields in search of proteins-based foods and other food items that were available. Another reason is the quasi-absence of natural enemies of *Bactrocera dorsalis* at the project sites. Moreover, there were a large number of alternate host plants around Christian and Adom farms (Ayikuma) serving as hiding places for the flies during the off season. The number increases rapidly when the main host mango, is ready and in abundance for flies to lay their

eggs and multiply. For instances, there were several cucumbers, melons, pepper and tomatoes farms observed a few meters within the study areas which could serve as alternative host for the flies. The high population build ups of fruit flies could also be attributed to poor management practices such as poor orchard sanitation where dropped fruit were not collected and buried on time. These dropped fruits served as a puparation medium for the larvae to grow in the soil for completing of their life cycle. The situation was different in Proper and Boko farms (Somanya) where the use of management strategies such as cattle to graze in the farms and even animal eating dropped fruits made the farm clean breaking the life cycle of the fruit flies. The result confirms the works done by Varela and Seif (2006), that sanitation combined with use of lures and traps as well as baits proved to be best package for the management of fruit flies.

Vayssières*et al*. (2009b) reported that abiotic factors such as humidity, rainfall and temperature were closely linked with fruit flies population and its dynamics. Christian and Adom farms (Ayikuma) had a relatively high humidity compared to Prosper and Boko farms (Somanya). The low relative average population density observed in Prosper and Boko farms (Somanya) was expected since these are situated in a savannah area which is different from that in the Greater Accra Region. This area is sparely wooded and hence there was low chance of finding hosts for the fly. This confirms the work by Israely *et al*. (2005) in their study on Metapopulation Spatial-Temporal Distribution Patterns of fruit flies in the Patchy Environment Israel, in which they found that the flies were distributed least in the drier parts of the study area. They attributed this to relatively slimmer chances of finding wild hosts of the flies in such an area. The large canopy covers of trees create a relatively high humidity in Christian and Adom farms (Ayikuma). Also these two farms were closer to the foothill of Akuapim Range and this also created a high humidity in those areas. This humid condition is

favourable for *Bactocera dorsalis*growth, development and mulitiplication hence its dorminance inChristian and Adom farms (Ayikuma).

Nboyine *et al*.(2012) also concluded that the Southern sector of the country has bimodal regime of rainfall.Rainfall makes the soil moist and thus provides some favourable conditions for eclosion of adults from their puparia. Fruit fly species that dorminated in the study areas was *Bactrocera dorsalis*, followed by *Zeugodacus cucurbitae*, *Ceratitis capitata* and *Ceratitis cosyra* in terms of abundance. However, most of the catches of *Bactrocera dorsalis* were made in March, April and May. This was in agreement with the work by Bagle and Prasad, (1983) where they used methyl eugenol to trap fruit flies and observed that the months of March, April, May and June recorded the peak period of catches of *Bactrocera dorsalis*. According to Rwomushana *et al*. (2006) the *Bactrocera dorsalis* thrives well in high temperatures and moist weather. Thus, more *Bactrocera dorsalis* were noticed during the months with high rainfall. This was the period of adequate rainfall. The first important rains and increasing humidity are important factors favouring its increase, especially as it coincides with the mango fruiting season. Mwatawala *et al*. (2006b) showed that *Bactrocera dorsalis* populations increased from the onset of the short rainny period onwards reaching maximum at this period. The relationship between the start of the rainy season with the increase of *Bacrocera dorsalis* and heavy mango losses, has also been observed in other West African countries (Vayssières*et al*., 2005). Around this same period, the general atmospheric humidity and temperature were also favourable for the growth and development of most insect species. Hence it was not surprising that *Bactrocera dorsalis* catches were high around this time.. Hagen *et al*. (1981) also observed that the most important factors, which contributed to population increase, were host availability and total monthly rainfall, which helped in the development of the flies.

Trap catches of flies after 7 weeks of baits application started to reduce steadily in all the four study areas in the treated plots. This observation had earlier been reportedby Pinero *et al*. (2010) that the bait sprays used, suppressed population of fruit flies for the first10 weeks of application.

Even though trap catches from treated plots tended to fall with time, those from control plots followed the same trend. This is because; the farmers in all the four farms were allowed to carry out their own treatments in their farms. *Bactrocera dorsalis* catches in all the treated plots in the four farms were high compared to the control plots, because treated plots had the lures and food baits whiles the control plot had only the lure. The high numbers could be attributed to the presence of methyl eugenol and food baits lures in the farms. Steiner *et al*. (1965) confirmed that combination of the lure and baits with toxicant had been successfully used in *Bactrocera dorsalis* eradication in other parts of the world.

The population of fruit flies remained relatively high even after harvest. This could be attributed to matured fruits of other varieties in other nearby farms and flies emerging from windfall fruit or mummies on trees. According to Puche *et al.* (2005) host fruit availability is the most important that bring about population fluctuation of fruit flies.

5.4 Non-target captures

A total of 2,605 non-target species of insects were captured during the pretreatment and the treatment periods. Most of the non-target organisms collected during the studies were attracted to methyl eugenol (Ekesi and Billah, 2006). The non-target species collected were from eight insect Orders: Coleoptera, Diptera, Blattodea, Lepidoptera, Areneae, Odonata Orthoptera and Odonata. Cuelure, Trimedlure and Terpinyl acetate were the second, third and

fourth in ranking respectively after methyl eugenol. The low number of non-target species attracted by these four attractants could be due to low number of fruit flies attracted to them. Most of the non-target species attracted to the trap could also be due to preying on the dead insects in the trap and they were knockdown by the killing agents DDVP. The non-target results obtained confirms that of Nboyine *et al*. (2012) which concluded that most of the non-targets were predators/parasitoids (Araneae, Blattodea, Hymenoptera and Odonata). Most of the Dipterans and some Coleopterans were decaying insect species which might be attracted due to decaying insects in the traps. The baits are fruit flies – specific since there was no record of capture of swallow tail butterfly or honey bee or fruit flies parasitoid in the traps. Since no swallow tail butterfly or honey bee were attracted to the traps it implies that the locally Spent Brewery Grain (Pito and Brukutu) and the two standards (GFFB and SUCCESS APPAT) can be incorporated into the fruit flies control programmes for effective results.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The main objective of this study was to evaluate the substitution of commercial components with cheaper, home-made ones to reduce crop losses as well as the costs of controlling fruit flies because commercially produced baits are too expensive or are unavailable. Even if they were imported, small scale farmers may not be able to procure it. Results from the study indicated that Spent Brewery Grain of Pito and Brukutu performed better than the control in reducing fruit attack by fruit flies. This implies that continuous use of Locally Spent Brewery Grain of Pito and Brukutu when integrated with other crop hygiene management practices such as farm sanitation can help control fruit flies attack. Spent Brewery Grain of Brukutu was superior to Spent Brewery Grain of Pito in controlling fruit fly damage. In Ghana there are a number of brewery grains across the country due to local drinks brewery industries in the country. If farmers are able to use the brewery grain and produce their own local protein bait, then the costs involved in fruit fly suppression and monitoring could be reduced, thereby enhancing the sustainability of fruit fly control activities in the country and it will be of immense benefit to low income mango farmers.

Bactrocera dorsalis, *Zeugodacus cucurbitae*, *Ceratitis capitata* and *Ceratitis cosyra* were the fruit flies species collected in the study areas. The most abundant was the *Bactrocera dorsalis* followed by *Zeugodacus cucurbitae*, *Ceratitis Capitata* and the least was *Ceratitis cosyra* in all the four farms. Christian farm (Ayikuma) recorded the highest catches of *Bactrocera dorsalis* followed by Adom farm (Ayikuma), Proper farm (Somanya) and Boko farm (Somanya). Summation of the entire individual relative fly density to plots in all the four farms indicated that Great Fruit Fly Bait (GFFB) plots recorded the highest fly density

followed by Spent Brewery Grains of Pito (SBGP), Control, Spent Brewery Grain of Brukutu (SBGB) and SUCCESS APPAT (GF-120) plots in that order. Christian farm (Ayikuma) obtained the highest relative fly density followed by Adom farm (Ayikuma), Proper farm (Somanya) and Boko farm (Somanya) in that order. The treatment period recorded the highest number of relative fly density than the pretreatment. This indicates that traps and bait attracted flies from other farms.

6.2 Recommendations

1. Locally spent brewery grain of both Pito and Brukutu should be used in Integrated Pest Management with other compatible methods such as orchard sanitation to reduce the menace of fruit flies on mango.
2. Since locally spent brewery grains are always available throughout the year, farmers should apply it in their farms twice a week to help reduce the population of fruit flies.
3. Since the cost involved in the preparation of local protein baits is not high, small-scale and mid-scale farmers should be encouraged to spray the locally-made protein baits area-wide so that the possibility of reinvasion is minimized in their farms instead of spot spraying in order to achieve a higher protection to the fruit.
4. Application of locally Spent brewery grain of Pito and Brukutu should be applied in the farm throughout the year whether fruit were present in the mango tree or not.
5. No alternative host crop should be planted within the farm for fruit flies during non-fruiting periods.

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APPENDICES

Appendix 1:Pictures of some milled local spent grain of Pito and the two standard products.

A: Grinding of SBGP

B: Spot spraying of bait

C : SUCCESS Appat (GF-120)

D: Great Fruit fly bait (GFFB)

Appendix 2: Data sheet for fruit fly species

Species of fruit fly	SBGP			SBGB			(GF-120)			(GFFB)			Control plot		
	R_1	R_2	T	R_1	R_2	T	R_1	R_2	T	R_1	R_2	T	R_1	R_2	T
Methyl eugenol trap															
Trimlure trap															
Terpinyl acetate trap															
Cue lure Trap															

Appendix 3: Trap data sheet for non-target species

Species of non-target	SBGP			SBGB			(GF-120)			(GFFB)			Control plot		
	R_1	R_2	T	R_1	R_2	T	R_1	R_2	T	R_1	R_2	T	R_1	R_2	T
Methyl eugenol trap															
Trimlure trap															
Terpinyl acetate trap															
Curelure trap															

Appendix 4: Pictures of some Tephritid flies captured by traps

Africa Invader fly, *Bactrocera dorsalis* (Hendel)

Melon fly, *Zeugodacus cucurbitae* (Coquillett)

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann)

Mango fruit fly, *Ceratitis cosyra* (Walker)

Appendix 5: Plots design and trap layout at Prosper Farms

SBGP	SBGB	GF-120	GFFB	CONTROL
1 2 3 4 1 3 2 4	4 2 1 3 2 1 3 4	4 2 1 3 4 1 2 3	1 2 4 3 3 1 4 2	3 2 1 4 2 3 4 1

Appendix 6: Plots design and trap layout at Boko Farms

SBGB	SBGP	GFFB	GF-120	CONTROL
2 1 3 4 1 2 4 3	3 4 1 2 3 4 1 2	2 4 1 3 4 1 2 3	1 2 4 3 2 1 5 3	3 2 1 4 2 3 4 1

NB:

Methyl eugenol, (ME) 1
Terpinyl acetate, (TA) 2
Trimedlure, (TML) 3
Cuelure (CUE) 4

Appendix 7: Plots design and trap layout at Christian Farms

CONTROL	SBGP	GF-120	GFFB	SBGB
2 1 3 4 1 2 4 3	3 2 2 4 1 3 4 1	3 2 1 4 2 1 4 3	1 2 4 3 3 1 4 2	1 2 4 3 2 1 4 3

Appendix 8: Plots design and trap layout at Adom Farms

GFFB	SBGP	SBGB	CONTROL	GF-120
1 2 3 4 2 1 4 3	4 2 1 3 2 1 4 3	4 2 1 3 4 1 2 3	1 2 4 3 3 1 4 2	1 2 3 4 4 2 3 1

NB:

Methyl eugenol, (ME) 1
Terpinyl acetate, (TA) 2
Trimedlure, (TML) 3
Cuelure (CUE) 4