PARASITISM BEHAVIOUR OF THE BRACONID WASPS, *COCCYGIDIUM LUTEUM* AND *COTESIA ICIPE* AND THEIR PERFORMANCE ON THE FALL ARMYWORM (*Spodoptera frugiperda)* LARVA.

BY

SAMUEL ADJEI MENSAH

(10805060)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL IN ENTOMOLOGY DEGREE.

AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE (ARPPIS)

UNIVERSITY OF GHANA,

LEGON

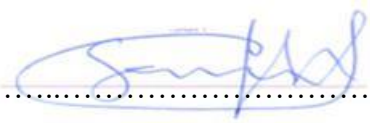
COLLABORATING DEPARTMENTS:

ANIMAL BIOLOGY AND CONSERVATION SCIENCE (SCHOOL OF BIOLOGICAL SCIENCES), AND CROP SCIENCE (SCHOOL OF AGRICULTURE), COLLEGE OF BASIC AND APPLIED SCIENCES.

DECEMBER, 2021

DECLARATION

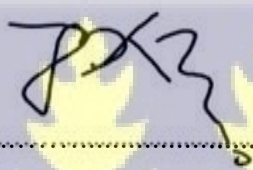
I hereby declare that this work is the result of my personal research and has not been fully or in part presented as a dissertation for a degree anywhere else. Works by other authors which served as a source of information, have been duly acknowledged by reference to the authors.



……

SAMUEL ADJEI MENSAH

(STUDENT)



……

DR. MAXWELL K. BILLAH

(SUPERVISOR)

……

DR. MILLICENT A. COBBLAH

(SUPERVISOR)

DEDICATION

I dedicate this thesis to the Almighty God, my dear mother Madam Patience Anum, and in memory of my late father, Mr. Emmanuel Mensah Sowah.

ACKNOWLEDGEMENTS

I express my profound appreciation to my project supervisors, Dr. Maxwell K. Billah for the immense financial assistance and advice throughout the study and Dr. Millicent A. Cobblah for her supervision and contribution to the thesis.

I also appreciate the assistance received from the Ghana Scholarship Secretariat for my second year tuition fees during the thesis work period. My gratitude also goes to Dr. Victor Clottey; Regional Coordinator, West Africa Centre, CABI for his financial assistance in my first year of study.

I further express my heartfelt appreciation to the Plant Protection and Regulatory Services Directorate (PPRSD) and Centre for Agriculture and Bioscience International (CABI) for the use of their biological pest control laboratory for the study.

My profound gratitude also goes to all lecturers of African Regional Postgraduate Programme in Insect Science (ARPPIS) who I approached at one point or the other for their academic guidance and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABBREVIATION	FULL MEANING
AND	(ATTACK- NO- DEFENSE). Parasitoid attacked (probed) but no defense (physical aggression) observed in Fall armyworm larva.
AD	(ATTACK-DEFENSIVE). Parasitoid attacked (probed) but Fall armyworm larva was defensive (physical aggression).
NA	(NO-ATTACK). No parasitoid attack (probe) on Fall armyworm.
FAW	Fall armyworm.

ABSTRACT

Fall armyworm is an invasive crop pest that has caused much damage to food crops since it invaded West Africa in 2016. *Coccygidium luteum* and *Cotesia icipe* were among the most promising Fall armyworm larval parasitoids scouted and identified in the West African sub-region. The study focus on making a choice on using either one, or both larval parasitoids in a Fall armyworm biological control programme in Ghana, as parasitoid populations of these species were different in East and West African sub-regions. The generation time, preference, parasitism levels, and a test for competition and its effect on Fall armyworm were conducted for both parasitoid species.

Coccygidium luteum recorded an average generation time of 30.1 days and maximum voltinism of 12 generations per year. *Cotesia icipe* recorded an average generation time of 16.9 days and maximum voltinism of 21 generations per year. *Coccygidium luteum* showed parasitism preference from newly emerged larva to 7 days old larva (1st to early 4th Fall armyworm larval instar). Four and five days old Fall armyworm larva (2nd and early 3rd larval instar) was ideal for higher parasitism, with a lower percentage mortality and higher percentage adult parasitoid emergence. *Cotesia icipe* showed parasitism preference from newly emerged larva to 4 days old larva (1st to 2nd Fall armyworm larval instar). One day, and two days old Fall armyworm larva (1st Larval instar) was ideal for higher parasitism, with lower percentage mortality and higher percentage adult parasitoid emergence. *C. luteum* recorded an average of 30 parasitism daily after two hours exposure (59.5 %), and a total of 149 parasitism in five days. *C. icipe* recorded an average of 24 parasitism daily, after two hours exposure (44. 7%), and a total of 112 in five days. Competition was found to exist between both parasitoid species as they exploit

the same host larva with an overlapping preference range, but the competition works synergistically in controlling Fall armyworm through increase in Fall armyworm mortality. *C. luteum* attacked largely day 0 to 7 days old Fall armyworm larva (1st to early 4th instar). *C. icipe* attacked day 0-4 day-old larva (1st to 2nd instar). Older Fall armyworm larva (5th instar and above) expressed a physical aggression defensive behaviour.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Study background

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) is an invasive species which originated from Americas and is currently present in many parts of Africa. It is a destructive agricultural pest which usually destroys crops by feeding on their leaves and stem, and even feed as deep into the corn (Marenco *et al.,* 1992). The Fall armyworm has a higher crop destroying tendency within the shortest time and also attack crops all year round. There is evidence of Fall armyworm resistance development to chemicals (Yu, 1991).

The Fall armyworm is a major crop pest which feeds on a variety of crops (polyphagous) and poses a threat to world food security. In Ghana, the Fall armyworm invasion has deprived many farmers of quality crop yield and bumper harvest (Day *et al.*, 2017).

Chemical control of the Fall armyworm has been the most used management practice. Some research institutes like Centre for Agriculture and Bioscience International (CABI) and the International Institute of Tropical Agriculture (IITA) in collaboration with other government institutions in West Africa have identified locally available Fall armyworm biological control agents. *Coccygidium luteum* and *Cotesia icipe* have been identified as two of the most abundant Fall armyworm larval parasitoids in Ghana and Benin (Abgoyi *et al.,* 2020). Further studies shows evidence of 89% reduction in the feeding rate of *C. luteum* parasitized Fall armyworm larvae (Agboyi *et al.,* 2019). The Ghanaian

government has shown kin interest in adopting biological control of the Fall armyworm in Ghana and has already initiated a nationwide biological control programme with emphasis on the most promising larval and egg mass parasitoids identified in Ghana. Currently the Plant Protection and Regulatory Services Directorate (PPRSD) of the Ministry of Food and Agriculture (MoFA) in collaboration with CABI is working on an egg mass parasitoid efficacy test on the Fall armyworm. *Coccygidium luteum* and *Cotesia icipe* are also promising larval parasitoid of great interest in the biological control of the Fall armyworm pest in Ghana. It is therefore prudent to conduct further studies on potential larval parasitoids intended to be used in Ghana. The presence of native Fall armyworm larval parasitoids in the West African sub region are key biological control agents which are intended to be used in augmentative biological control. Understanding the ecological interactions between the Fall armyworm and their environment which includes the beneficial biological control agents plays an important role in the success of a biological pest control programme (Xu *et al.*, 2013).

1.2 Problem statement

The damage Fall armyworm poses to crop yield loss and food security cannot be over emphasized. Fall Armyworm (FAW) is estimated to cause yield losses in a range from 8.3 to 20.6m tonnes per annum representing economic loss between US$2,481m and US$6,187m in Africa if not managed properly (Abrahams *et al.*, 2017). Fall armyworm related average maize yield loss in Ghana as at 2018 was 45%, with lower and upper extremes of 22% and 67%, respectively. This accounts for an estimated US$418.8m economic loss annually (Abrahams *et al.*, 2017). Measures put in place to curb the

menace are more chemically inclined and have not yielded a very effective control. This has enhanced the establishment and increase in Fall armyworm pest population resulting in further spread of the pest to many more countries in Africa (Cock *et al.*, 2017).

The government of Ghana spends over US$5.02m for distribution of pesticides and monitoring of Fall armyworm (SRID, 2018). The borrowing behaviour of Fall armyworm larvae into the plant and soil, while feeding and pupating respectively makes Fall armyworm pest control not very efficient **(**Kumela *et al.*, 2018**)**. Adaptive potentials of Fall armyworm have also enhanced their resistance to insecticides and transgenic corn hybrids making control really difficult (Julio *et al.*, 2017). The effect of chemical on human health, the environment and biodiversity sustainability cannot be disregarded and this calls for the development of a sustainable and eco-friendly control method (Boxall, 2004**).** Biological control is one of the safe and effective pest control strategies. Several parasitoids of FAW including larval parasitoids like *Coccygidium luteum* and *Cotesia icipe* were identified in Ghana. However, little is known about their behaviour, particularly the interaction between different parasitoid species identified.

Intrinsic and extrinsic interspecific competition mechanisms are known to exist among the family Braconidae (Xu *et al.*, 2013). Several parasitoids have been found in Ghana, causing up to 75% parasitism to FAW larvae (Agboyi *et al.*, 2019). Amongst them the braconid wasps, *Coccygidium luteum* and *Cotesia icipe* were collected in Ghana causing up to 17% and 1% Fall armyworm parasitism, respectively. Unlike the case of Ghana, Eastern African countries (Kenya and Tanzania) have more *C. icipe* than *C. luteum* with less than 10% Fall armyworm parasitism (Fiaboe *et al.*, 2017; Sisay *et al.*, 2018). The differences in *C. icipe* and *C. luteum* abundance in the two African sub regions might be

due to the inter-specific competition among parasitoids which have been reported to affect the parasitoids population dynamics (Xu *et al.*, 2013). The success of released parasitoids in a biological control programme could be affected by the intense competition between parasitoids for resources and may alter the intended results (Wang *et al*., 2008; Cabello *et al.*, 2011). This calls for curiosity to understand what accounts for the contrasting abundance and level of parasitism between the two parasitoids in the different sub regions.

1.3 Justification

Augmentative biological control programme is always based on mass rearing of biological control agent, with a deep knowledge on its potential to control the target pest, as well as its interaction in the ecology. In-depth knowledge on parasitoid interactions in the environment and successful use in FAW biocontrol will drastically minimize the excessive use of chemicals, including very hazardous and unregistered ones by farmers with adverse effects on human and environment, which is a major worry for the sustainable management of Fall armyworm.

The behavioural adaptations of the Fall armyworm must be taken into consideration before choosing a control method. More often than not, the early larval instars are not visible to the ordinary farmer. The farmer only gets to know of an infestation when the larvae are at the late instars, where they feed faster on large masses of the crop. At this stage, damage and presence of the Fall armyworm are visible. Unfortunately at this stage of infestation, farmers might have lost most of their crops. Pesticides though lethal to

Fall armyworm, might not be effective in killing and controlling the Fall armyworm population, this is due to the fact that the larvae bore deep into the whorl, stem, and cob at this stage of infestation (Sparks, 1979), and may not have frequent contact with the pesticides. This phenomenon reduces the probability of killing the larvae by the pesticide, though lethal.

On the other hand, the frequent contact with chemicals over a long period of time may lead to increase in resistance to the pesticides by the Fall armyworm. Pesticides are synthetic compounds and have a broad spectrum, killing non targeted insects that are important in ecological processes (Margni *et al.*, 2002). Synthetic chemicals stay longer in the environment and may lead to chemical accumulation and toxicity in living organisms. This makes them unfriendly to the environment (Mahmood *et al*., 2016).

Among parasitoids found in Ghana, the three most abundant egg-larval and larval parasitoids belong to the family Braconidae. They include the egg-larval parasitoid *Chelonus bifoveolatus* and the larval parasitoids *Coccygidium luteum* and *Cotesia icipe* (Agboyi *et al*., 2020) Cotesia icipe was the most abundant larval parasitoid found in the study (Agboyi *et al.*, 2020). Further study also proved *C. luteum* cause a drastic reduction of maize leaf feeding in parasitized Fall armyworm (Agboyi *et al.*, 2019). Findings from these research provides much information to focus more on the most abundant parasitoids to test their efficacy in controlling Fall armyworm in the West African sub regions and also understand the dynamics in their reproductive and interactive behaviour, hence the importance of carrying out this study on their interactions with other parasitoids and Fall armyworm. Knowledge of parasitoid parasitizing capacity,

interaction, and larval preference will provide baseline information on choosing either or both parasitoids for a biological control programme in Ghana.

1.4 General objectives

The major purpose of this study is to provide enough scientific knowledge on the parasitism behaviour of *C. luteum* and *C. icipe* and their interaction that could help develop an effective augmentative biological control protocol against Fall armyworm in Ghana. The results obtained will be considered a base line data to inform the necessity to consider one or both of the two parasitoids in an augmentative biological control programme in Ghana, and also provide data for the estimated number of parasitoid actives which should be mass reared and released to effectively reduce the Fall armyworm pest damage bellow economic injury level.

1.5 Specific objectives

The specific objectives of this research are to:

1. Identify Fall armyworm larval instars preferred by *C. luteum* and *C. icipe*.
2. Assess the parasitism potential of *C. luteum* and *C. icipe* on Fall armyworm larvae
3. Study the life span of *C. luteum* and *C. icipe.*
4. Study the interaction (synergy/competition) between *C. luteum* and *C. icipe* in the presence of FAW larvae.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background information of Fall armyworm

Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is an invasive agricultural pest of tropical-subtropical origin in the Americas (Sparks, 1979). Although its larvae feed on a variety of plants, it is predominantly a pest of maize but has a wide host range and is capable of feeding on over 80 plant species, periodically causing significant economic damage to rice, sorghum, millet, soybean, wheat, alfalfa, cotton, turf, and fodder crops (Montezano *et al*., 2018) . The Fall armyworm adult is nocturnal in its feeding and mating activities. Females may mate severally and using pheromones as male attractants. Larvae are known to complete 6 instars and pupate in the soil (Sparks, 1979).

2.1.1 Fall armyworm life cycle and biology

The Fall armyworm is a holometabolous insect with four life stages; the egg, larva, pupa and adult. The Fall armyworm adult is a nocturnal moth. Adults launch early evening movement near suitable host plants for feeding at dusk, where oviposition and mating takes place. Eggs are laid in masses and protected by a dense covering of scales. Egg masses contain a few to hundreds of eggs which hatch in 2-4 days with 22 to 26 °C mean temperature. Fall armyworm larvae are the life stage that causes much economic damage to crops. The larvae feed on the egg shell as they emerge and initiate feeding on the host

plant, and then continue to consume foliage until they have completed 6 instars and pupated (Sparks, 1979).

The first 3 instar are relatively small and require less than 2% of the total Fall armyworm older larvae foliage consumed. The 6th-instar enters to the ground and pupates 1 to 4cm below the soil surface, depending upon soil texture, moisture, and temperature. According to Vickery (1929) length of pupal period varies from 7-37 days, again depending on soil mean temperatures ranging from 15 to 29 °C .When the insects emerge from the pupal cases, they find their way to the soil surface where they grip plants or plant debris, bloat their wings, and become adult in appearance (Sparks, 1979).

2.1.2 Distribution of Fall armyworm

Fall armyworm species has a tropical-subtropical origin in the Western Hemisphere (Johnson, 1987). Wiltshire (1977) suggested that Fall armyworm had become well settled in other parts of the world, including Israel. He also indicated that morphological races of Fall armyworm may exist; genital comparisons of specimens from Israel, Brazil, the British Museum, and drawings from museum indicated that the Israeli Fall armyworm populations originated from a Caribbean-USA source and not South America (Brazil).

Until 2015, the Fall armyworm pest has not been reported in any other part of the word except in America. In 2016, it was recorded in Africa causing serious damage on maize crop (Goergen *et al*., 2016; Abrahams *et al*., 2017). Previously, Fall armyworm was not recorded to have been found outside the Americas but its two strains (rice and corn) have now appeared in Africa and are rapidly distributed throughout the tropical and subtropical

regions (Cock *et al.*, 2017) (Fig. 1). Fall armyworm is capable of migrating long distances on prevailing winds, but it can also breed continuously in areas that are climatically suitable. West Africa was the first African sub region to record Fall armyworm in early 2016 (Goergen *et al*. 2016; Cock *et al.* 2017).

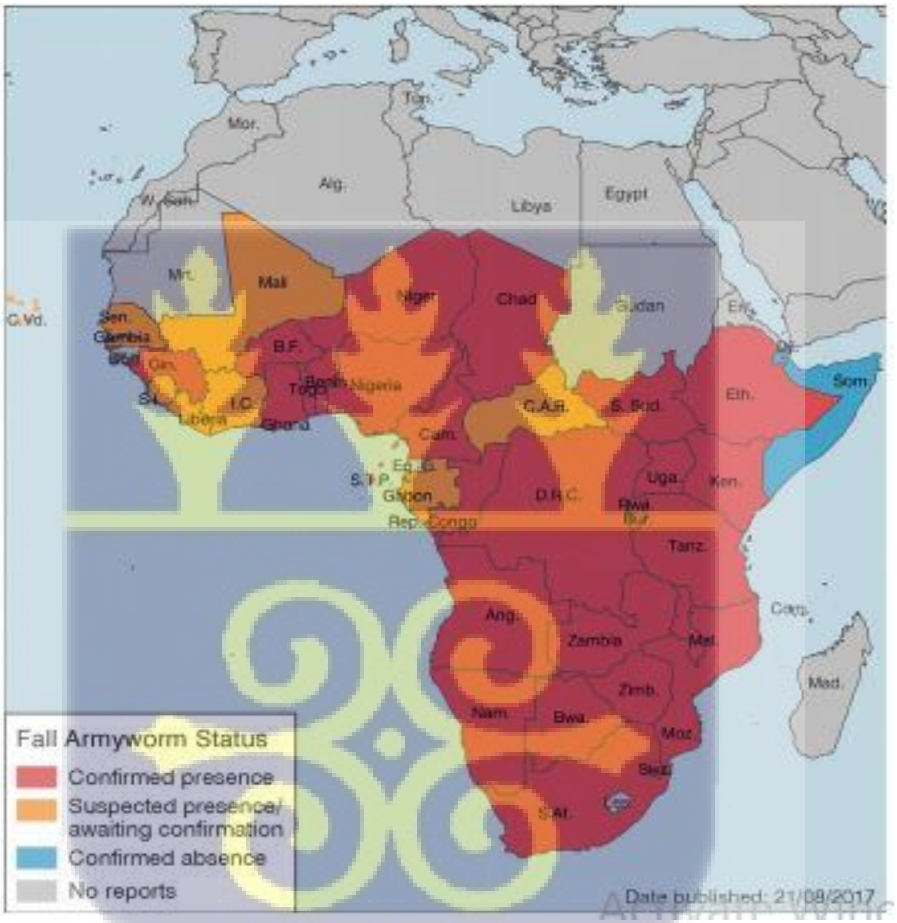


Figure 1.0: Fall armyworm distribution in Africa (Day *et al.*, 2017).

2.1.3 Morphological identification and development of Fall armyworm.

Fall armyworm Adult.

The adult Fall armyworm has a wingspan between 32 and 40 mm. The Male moth, typically have a larger portion of forewing dark gray and with brown patches, with triangle-like white markings at the tip and close to the middle of the wing (Fig. 2). The female forewings are less uniquely marked, usually uniform grayish brown to brown in colour (Fig. 2). The hind wing is silver-white or pale-white with a thin dark wing edges in both male and female (Sparks, 1979; Capinera, 2002).

After a pre-oviposition period, the female lays most of its eggs within the forth to fifth days of life, and occurs up to three weeks in some cases. The average lifespan of the adult is estimated to average about 10 days, with about 7 to 21 days range (Capinera, 2002).

A.  **B.**

Figure 2.0: Fall Armyworm adults. A = Male, and B = Female. Source: Agric. Research Council, South Africa (2017).

Fall armyworm eggs.

The Fall armyworm egg is dome-shaped with base flattened. A single egg measure about 0.3 mm in height and 0.4 mm in diameter. The total number of individual eggs in an egg mass varies but is usually 100 to 200, and total egg production by a female average about 1500 and over 2000 at maximum (Fig. 3). The female Fall armyworm lays a layer of grayish scales between and on top of the egg mass during oviposition, which gives the egg mass a moldy or whitish appearance. The egg usually hatches in two to three days during the summer months (Ashley *et al.*, 1989).

Figure 3.0: Fall armyworm egg mass with whitish furry or moldy appearance. Source: Agric. Research Council, South Africa (2017).

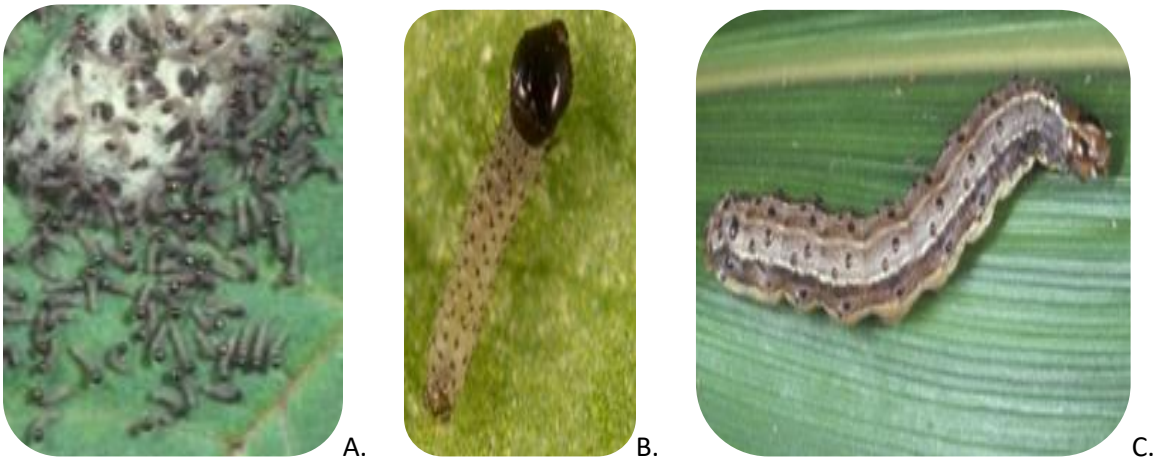
Fall armyworm larva.

Fall armyworm has a total of six (6) larva instars (Fig. 4). Larvae head capsule widths measures about 0.35, 0.45, 0.75, 1.3, 2.0, and 2.6 mm, for larval instars 1 to 6, respectively. Larval lengths of instars 1 to 6 measures about 1.7, 3.5, 6.4, 10.0, 17.2, and 34.2 mm, respectively (Pitre and Hogg, 1983; Capinera, 2002).

First to second larval instars are pale green with a black head. The head usually turn pale orange in the second instar. Third larval instar has a brownish dorsal surface integument and a faint white lateral lines forming on its surface (Pitre and Hogg 1983; Capinera, 2002).

Head usually turns reddish brown in the fourth to the sixth instars and the brownish integument has a white sub-dorsal and lateral lines. There are usually dark elevated spots which occur dorsally on the body with spines. The frontal area of the head of the mature larva is prominently marked with a white inverted "Y" with a rough or granular larva epidermis texture when examined closely (Capinera, 2002) (Fig. 5).

The larva however does not feel rough when touched, because it lacks the microspines. Besides the usual brownish colour of the Fall armyworm larva, the larva may be mostly green dorsally. The dorsal elevated spots appear pale in the green form rather than dark. Mean development time of the Fall armyworm larva was determined to be 3.3, 1.7, 1.5, 1.5, 2.0, and 3.7 days for first to sixth instar, respectively, when reared at 25ºC (Pitre and Hogg, 1983).



A. B. C.

Figure 4.0: Different stages of the Fall Armyworm. **A**. = freshly emerged larvae **B**. = first instar, and **C.** = sixth instar larva (Source: Agric. Research Council, South Africa (2017).

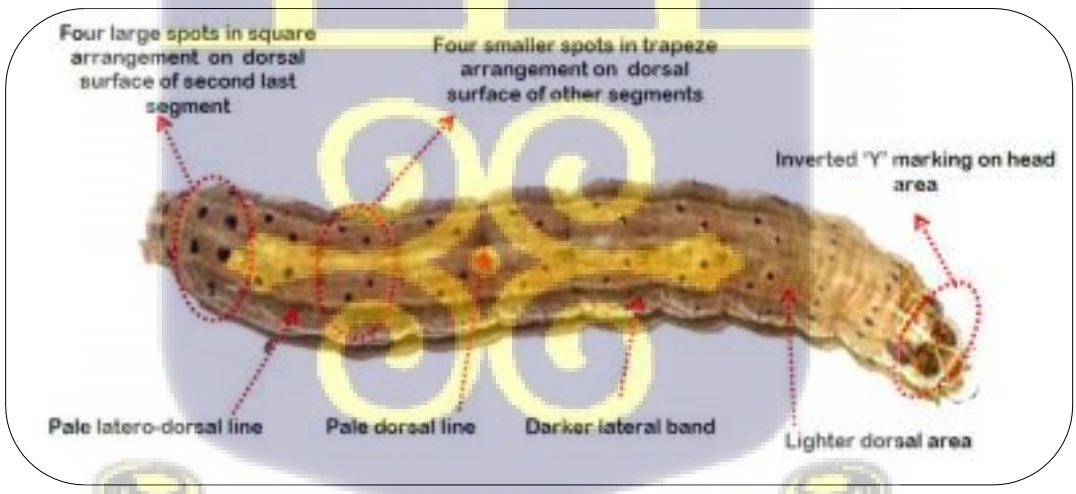


Figure 5.0: Annotated image showing morphological characteristics of Fall armyworm larva. Source: Agric. Research Council, South Africa (2017).

2.1.4 Damage and economic implications.

Fall armyworm causes damage to almost all the parts of its host plants (Fig. 6). The first instars larvae scrape the green fresh leaf surfaces when feeding. The second to sixth larval instars cause defoliation of leaves in the shortest possible time by chewing with a much stronger and larger mouth part (Pannuti *et al.*, 2016). This reduces plant green foliage and as a result, reduces photosynthesis which then cause lower crop yield (Heidari, 2012). Older larvae feed their way into the stems and the corn, which leads to broken stems and unwholesome corn. This causes a high significant economic loss to farmers and nations at large (Cruz, *et al.*, 1999).

Spodoptera frugiperda is presumed to be a crucial pest of corn in Brazil, one of the largest corn producers in the world after the USA and China (Fritz *et al*., 2015), and also in most of the African countries. Maize is the most extensively grown crop in Africa and essential for around half the continent's people. Fall armyworm threatens food security in Africa, where maize is a staple food (Hailu *et al.*, 2018; Harrison *et al*., 2019). In Mozambique, 21–90% of African households depend on maize for daily subsistence (Dias, 2019; Canico, 2020). It is cultivated across diverse agro-ecological zones, and over 200 million people are reported to depend on the maize for food security (Ranum *et al*., 2014).

Essentially, maize accounts for about half of the calories and protein consumed in eastern southern and eastern parts of Africa, and one-fifth in West Africa (Macauley, 2015). In July 2017, a household socio-economic survey was conducted in Ghana and Zambia. The survey investigated farmers' perception of losses peculiar to Fall armyworm over the full growing maize season. According to the survey results, the estimated national mean loss

of maize in Ghana was 45% (range 22–67%), and in Zambia 40% (range 25–50%) (Abrahams *et al*., 2017; Cock *et al*., 2017).

According to data collected from Ghana and Zambia, the potential impacts on national yield and revenue in ten other major maize cultivating countries was estimated, with the assumption that the Fall armyworm will spread throughout all areas where it is predicted to be established (Abrahams *et al*., 2017). Fall Armyworm could potentially cause corn yield losses in a range from 8.3 million to 20.6 million tonnes in 12 African countries per annum, if not managed properly. The value of these losses is estimated at between US$2,481 million and US$6,187 million (Abrahams *et al.*, 2017).

A. B.

Figure 6.0: Fall Armyworm damage to corn ear by larvae **(A)**, leaves and stem **(B).** Source: Agric. Research Council, South Africa (2017).

2.1.5 Fall armyworm management.

Before Fall armyworm invaded Africa, it was projected that over 97% of smallholder farmers did not use any synthetic chemical for maize production pest management

(Kansiime *et al.*, 2019). That narrative however, was not the case after the immediate detection of Fall armyworm, because governments in many countries started distributing synthetic insecticides as an emergency response (Kumela *et al.*, 2018; Hruska, 2019; Sisay *et al.*, 2019). Farmers did not have access to meticulous information from agricultural extension officers on which pesticide to apply and how and when to apply, they mostly decided on their own, which lead to indiscriminate application of insecticides. The continuous and irresponsible application of harmful insecticides by farmers without adequate training on pesticides application and management are known to promote the development of resistance of Fall armyworm to these insecticides as reported in Puerto Rico and Mexico (Gutiérrez-Moreno *et al.*, 2019).

Pesticide application

Diverse forms of pesticides have been used for crop protection in several decades. Pesticides aid crops protection; however, they also impose a detrimental impact on the environment (Geiger *et al.*, 2010). Pesticides usage is a major concern for sustainability of environment and global stability (Margni *et al.*, 2002). The dangers related with pesticide use have exceeded their beneficial effects, where pesticides with wide target range kill non-target plants and animals along with the targeted ones of interest. Some pests develop genetic resistance to pesticides over a long period of time when exposed to them (Delaplane, 2001).

Glyphosate in herbicides increases vulnerability of plants to diseases (Brammall and Higgins, 1988) and may lead to seed quality reduction (Wagner *et al.*, 1995). Low doses of sulphonamides, sulfonylureas, and imidazolinones have a destructive impact on the

productivity of non-target crops, natural plant communities and wildlife (Fletcher *et al*., 1993). According to World Health Organization, each year, about 3,000,000 cases of pesticide poisoning and 220,000 deaths are reported in developing countries (Lah, 2011).

Fall armyworm biological control.

Biological control is the use of organisms to manage or interfere with the population increase and damage caused by a targeted pest species or the intentional manipulation of populations of living beneficial organisms in order to check the population of pests (Flanders, 1950). Successful biocontrol programmes normally reduce the abundance of the pest significantly.

Some biological control organisms do not reduce the abundance of a target pest but prevent the damage caused by the pest by reducing feeding on valued crops (Lockwood, 2000).

General approaches implored in biological control organisms usage are: 'Classical' biocontrol which targets a non-native pest with one or more species of biocontrol agents from the pest's native country or distribution range; 'Neoclassical' approach targets indigenous pests with non-native biological control agents; 'Conservation', 'Augmentation' and 'Inundation' approaches maintain or increase the abundance and impact of biocontrol agents that are already present, and in many cases indigenous to the area. Classical biocontrol is by far the most common approach for plant pests. Conservation typically deals with maintenance, manipulation and protection of indigenous biological agent environment to enhance their survival and establishment. Conservation and augmentation approaches show great promise on their own and

especially for enhancing the impacts of classical biocontrol as researchers show much interest in managing to maximize native biological diversity in invaded ecosystems (Newman *et al.*, 1998).

2.2 Fall armyworm parasitoids

There are several natural enemies of Fall armyworm insect pest word-wide, but parasitoids are the most used in biological control of the Fall armyworm due to their wider diversity and distribution (Sisay *et al*., 2018; Agboyi *et al.*, 2020; Caniço *et al.*, 2020). Parasitoids are insects that deposit their eggs in or on host insects. The young parasitoid larva feeds on the host when it hatches from the egg and results in host (pest) death.

2.2.1 Fall armyworm parasitoid diversity, distribution and abundance.

Fall armyworm has several natural enemies, either native or exotic that attacks it. These natural enemies include parasitoids and entomopathogenic fungi (Lezama-Gutiérrez *et al.*, 2001; Thomazoni *et al.*, 2014; Agboyi *et al*., 2020) which target different development stages (Dequech *et al.*, 2013; Ruiz-Najera *et al.*, 2007; Hay-Roe *et al.*, 2016) causing significant mortality and reduction of crop damages. Several different parasitoid species of Fall armyworm have been observed and identified to occur in its native geographical areas (Ashley *et al.*, 1986; Hoballah *et al*., 2004).

Fall armyworm parasitoid complex belonging to families Braconidae, Eulophidae, Ichneumonidae, and Tachinidae have been reported in Mexico and Florida (Molina-Ochoa *et al*., 2001; Hoballah *et al*., 2004; Ruiz-Najera *et al*., 2007; Rios-Velasco *et al*.,

2011; Hay-Roe *et al*., 2016). In Honduras, larval parasitoids were found to be the most occurring natural enemies of the Fall armyworm (Wheeler *et al*., 1989). In Africa, several hymenopteran parasitoids attacking different stages of Fall armyworm have been reported in many countries such as Benin, Côte d'Ivoire, Ethiopia, Ghana, Kenya, Niger, Nigeria South Africa ,Tanzania, and Togo (Sisay *et al*., 2018; Kenis *et al*., 2019; Agboyi *et al*., 2020).

A complex of egg, egg-larval, larval, and larval-pupal parasitoids of Fall armyworm including *Telenomus remus* Nixon (Hymenoptera: Platygastridae), *Trichogramma* sp., (Hymenoptera: Trichogrammatidae) *Chelonus bifoveolatus* Szépligeti (Hymenoptera: Braconidae), *Coccygidium luteum* (Brullé) (Hymenoptera: Braconidae), *Cotesia icipe* Fernandez-Triana and Fiaboe (Hymenoptera: Braconidae), *Meteoridea cf. testacea* (Granger) (Hymenoptera: Braconidae), *Charops* sp. (Hymenoptera: Ichneumonidae), *Metopius discolor* Tosquinet (Hymenoptera: Ichneumonidae), *Pristomerus pallidus* (Kriechbaumer) (Hymenoptera: Ichneumonidae), and *Drino quadrizonula* (Thomson) (Diptera: Tachinidae) were reported in Ghana and Benin (Agboyi *et al*., 2020). Different parasitoid species including *Bracon* sp. (Hymenoptera: Braconidae), *Anatrichus erinaceus* Loew (Diptera: Chloropidae), and an unidentified tachnid were also reported in Ghana (Koffi *et al*., 2020).

Parasitoid surveys conducted in Benin, Ghana, Kenya, and Tanzania showed evidence of difference in the sampled population of *Coccygidium luteum* and *Cotesia icipe.* The braconid wasps, *C. luteum* and *C. icipe* were more in Ghana causing up to 17% and 1% Fall armyworm parasitism, respectively (Agboyi *et al*., 2020). Unlike the case of Ghana, Eastern African countries (Kenya and Tanzania) have more *C. icipe* than *C. luteum* with

less than 10% Fall armyworm parasitism (Fiaboe *et al*., 2017; Sisay *et al*., 2018). The differences in *C. icipe* and *C. luteum* abundance in the two African sub regions might be due to the inter-specific competition among parasitoids which have been reported to alter the parasitoids population dynamics (Xu *et al*., 2013).

2.3 *Cotesia icipe* Fernandez-Triana and Fiaboen.

Cotesia icipe is a solitary Microgastrinae larval endoparasitoid wasp which belongs to the order Hymenoptera and family Braconidae and of much importance for the biological control of lepidopteran pests in Africa (Boisduval, 1833; Agboyi *et al*., 2020). The *Cotesia* genus is the second largest, with about 300 species described worldwide (Yu *et al*., 2016).

The Microgastrinae subfamily is reported to be the principal group of parasitoid wasps that parasitize insect pest larvae (Whitfield *et al*., 1997). Microgastrinae comprises more than 2700 described species, which include *Cotesia icipe* and several other undescribed species (Rodriguez *et al*., 2013; Yu *et al*., 2016).

Female was reported by researchers at the International Centre of Insect Physiology and Ecology (icipe) Kenya, to prefer ovipositing in second instar host larvae, in this case *Spodoptera littoralis* and *S. exigua* with mean development time from egg to adult in 14 days (Fiaboe *et al*., 2017) unlike *Cotesia marginiventris* (Cresson) which is also a solitary is reported to prefer larva instar 1 for oviposition and later emerge out of 4th instar Fall armyworm larva (Cave, 1995).

Cotesia icipe has been collected and reported to have emerged from Fall armyworm in some African countries including many in the West African sub region like Ghana and Benin (Agboyi *et al*., 2020).

Cotesia icipe was reported to have differentially accepted immature stages of Fall armyworm. The acceptance of 1st and 2nd instar Fall armyworm larvae for oviposition was significantly higher with more than 60% parasitism. No oviposition was recorded in the 5th and 6th larval instars. Female parasitoids were dissected and egg-load varied significantly with wasp age, with six-day-old wasps having the highest number of mature eggs. Newly emerged wasps (day 0) had a substantial number of eggs (<100), egg-load varied remarkably with the wasp's age (P = 0.001). Six-day-old wasps had the highest complement of mature eggs. Although egg load declined for the nine-day-old wasps, it was still higher than that for zero and three-day-old wasps. Ovigeny index of *C. icipe* was 0.53. The study was conducted in the laboratory at 25 ± 2˚C, 60–70% RH and a photoperiod of 12L: 12D. The moths and parasitoids were provided with 20% honey solution with a moistened cotton wool ball placed in a Petri dish (8.6 cm in diameter) (Mohamed *et al.*, 2021).

2.3.1 Morphological identification.

Subfamily Microgastrinae

Microgastrinae is a subfamily of Braconidae (Hymenoptera). Many morphological features enhance the identification and recognition of Microgastrinae. The most prominent and easy characters are: possession of an antenna with 16 flagellomeres with

the exception of a new genus and species discovered in New Zealand, *Kiwigaster variabilis*, which is unique amongst all microgastrines, because females and male have antenna with 18 and 17 flagellomeres, respectively; forewing typically with no readily visible veins on apical third of the wing (see red rectangle; Fig. 7). Also, the first metasomal segment has a strongly defined mediotergite without spiracles. The spiracles are situated on separate laterotergites (see red arrow on Fig. 7). Microgastrines have a relatively short metasoma (abdomen of other groups of insects) as compared with other groups of Braconidae (Moghaddam *et al*., 2021).

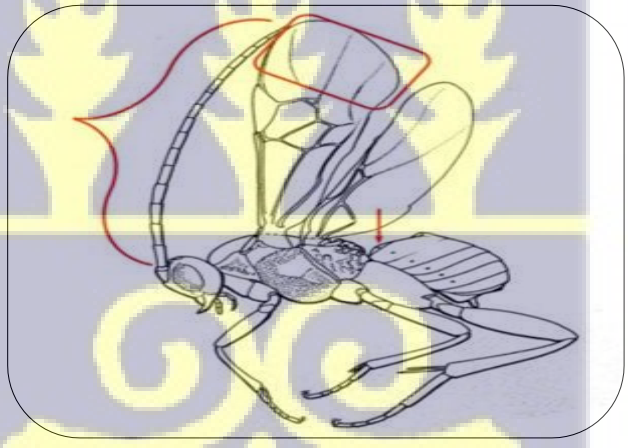


Figure 7.0: General morphological features used in identifying the subfamily Microgastrinae. (Source: Moghaddam *et al*., 2021).

2.3.2 General diagnosis of *Cotesia icipe* Fernandez-Triana and Fiaboe

The central part of metasomal mediotergite 3 has a dark brown to black colour with the lateral side yellow. The hind legs are mostly yellow, with the exception of the metacoxa, which is usually black in colour, with a little yellow spot on apical 0.1 and brown spots

on apical 0.1 of meta-femur (dorsally). The apical 0.1 of meta-tibia is dark brown, and metatarsus entirely dark brown. The tegula and humeral complex are yellow (Mason 1981; Karlsson and Ronquist 2012).

The forewing membranous with most veins brown. Besides colouration, *C. icipe* has scuto-scutellar sulcus with 8 carinae. Metasomal mediotergite 1 are almost parallel-sided with a slight widening towards posterior margin; metasomal mediotergite 2 is relatively small, squire-like and do not cover the entire surface of the tergum. Metasomal mediotergite 3 is 1.3 times longer than metasomal mediotergites 2 lengths (Mason 1981; Karlsson and Ronquist 2012; Fernandez-Triana *et al*., 2014; Fiaboe *et al*., 2017). *Cotesia* species are many and diverse, but the combination of characters described above is unique for *Cotesia icipe* identification.

A positive correlation is known to exist between parasitoid body size and host body size, especially in solitary species. Development time is longer for parasitoids attacking larger hosts and longer for koinobionts, which may have delayed development (Blackburn, 1991a). As demonstrated in the principle of minimizing time spent in vulnerable life-history stages (Stearns, 1992), it is expected that development time would be respectively shorter in parasitoids exposed to greater mortality risks, in this case parasitoids in smaller larval host (Blackburn, 1991a).

2.3.4 Description of species

Female has brown dorsal metasoma, except for metasomal mediotergite 3 (which is centrally brown and laterally yellow). Laterotergites and sternites are mostly yellow, with

brown hypopygium (Fig. 8). The length of antennal flagellomeres 2 is 1.78 - 2.00 times longer than the length of antennal flagellomeres 14. Metafemur length is 3.60 - 3.80 times wider than metafemur width. The inner spur length of the metatibia is 1.17 times longer than the outer metatibia spur length and inner metatibia spur length 0.52 times longer than first segment of metatarsus length (Fernandez-Triana *et al*., 2014; Fiaboe *et al*., 2017).

The metasomal mediotergite 1 is entirely engraved with coarse punctures and a polished knob at the centrally part of the posterior margin and slightly widened towards posterior margin (width at posterior margin 1.1 - 1.2 times wider than width at the anterior margin). Metasomal mediotergite 1 length is centrally 1.5 - 1.7 times wider than its width at the posterior margin, while metasomal mediotergite 2 is entirely sculptured with coarse punctures along all margins and longitudinal striation centrally having a more or less rectangular shape. The width at posterior margin is 2.0 times its length centrally. Metasomal mediotergite 3 have a smooth surface, with rows of setae that are denser on posterior half of tergite and central length 1.3 times longer than metasomal mediotergite 2 (Fernandez-Triana *et al*., 2014; Fiaboe *et al*., 2017).

Ovipositor sheaths are 0.16–0.19 times longer than metatibia length and measures 0.12–0.15 mm. *Cotesia icipe* has a body length of 2.20 - 2.50 mm and forewing length of 2.20–2.50 mm. The metafemur has a length of 0.65 mm and 0.17–0.18 mm width. Metatibia length is 0.76–0.80 mm with inner spur length of 0.21 mm and outer spur 0.18 mm. The first segment of metatarsus measures 0.40 mm in length. Metasomal mediotergite 1 measures 0.35–0.37 mm in length centrally, with anterior margin width at 0.19 mm, attaining a maximum width of 0.25 mm.

The metasomal mediotergite 1 width at posterior margin is 0.22–0.24 mm. Metasomal mediotergite 2 width at posterior margin is 0.32 mm, with a central length of 0.16 mm. Metasomal mediotergite 3 measures 0.21 mm centrally. The length of antennal flagellomeres 1, 2, and 3 are 0.17–0.19 mm, 0.16–0.18 mm, and 0.16–0.17 mm, respectively. Lengths of antennal flagellomeres 14, 15, and 16 are 0.09 mm, 0.08 mm, and 0.10 mm, respectively. Generally, males look morphologically similar to females, but with darker metasoma dorsally. Males sometimes have an entirely dark-brown to black metasomal mediotergite 3 (Fig. 9) (Fernandez-Triana *et al*., 2014; Fiaboe *et al*., 2017).

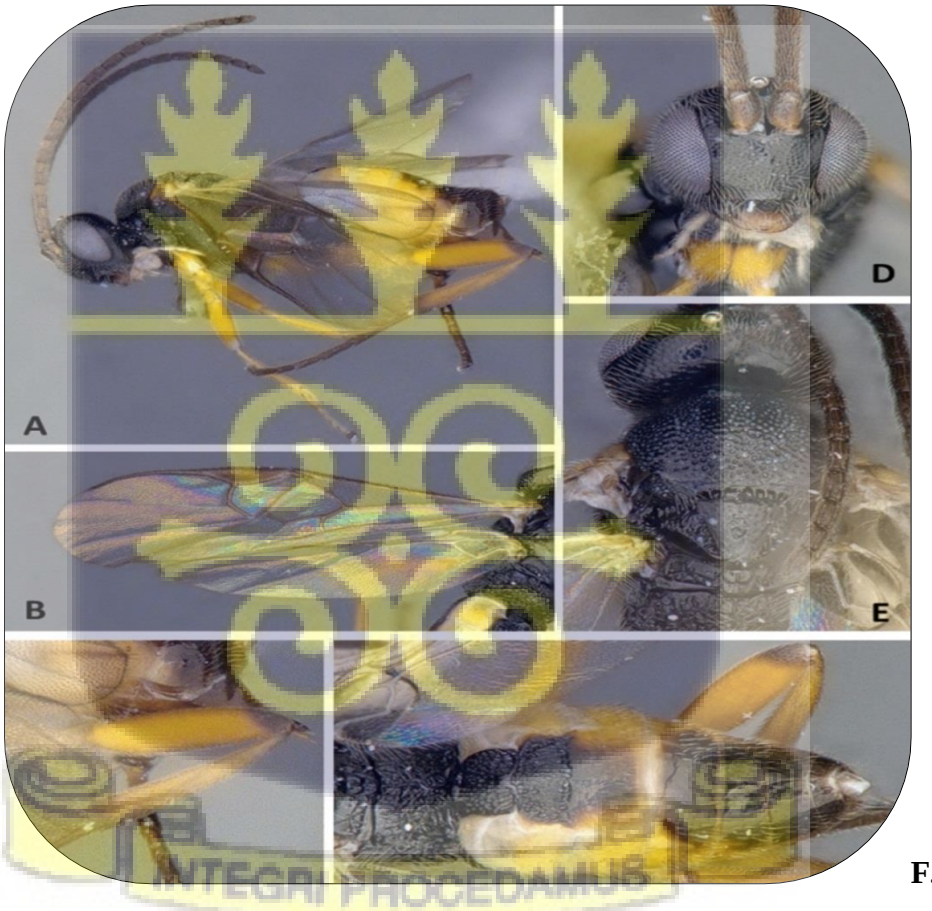


Figure 8.0. Female *Cotesia icipe;* (**A**) Habitus lateral, (**B**) Wings, (**C**) Hind leg, ovipositor and hypopygium, (**D**) Head frontal, (**E**) Mesosoma dorsal and head, and (**F**) metasoma dorsal. (Source: Fiaboe *et al*., 2017).

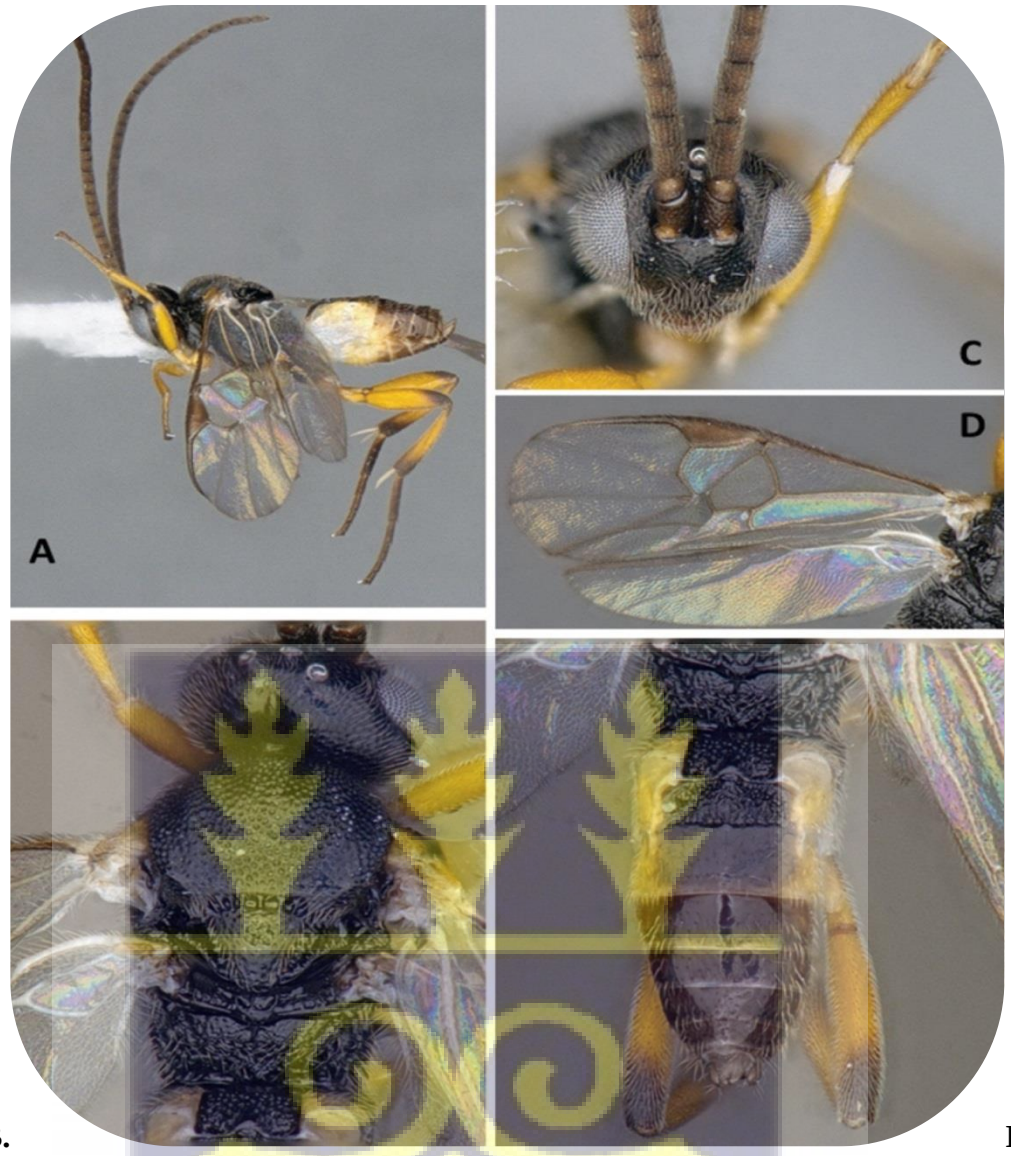


Figure 9.0. Male *Cotesia icipe;* (**A**) Habitus lateral, (**B**) Mesosoma dorsal and head, (**C**) Head frontal, (**D**) Wings, and (**E**) metasoma dorsal and propodeum. (Source:Fiaboe *et al*., 2017).

2.4 Coccygidium luteum (Brullé)

Coccygidium luteum (Brullé) is a solitary koinobiont parasitoid which belongs to the family Braconidae and sub-family Agathidinae, which consists of more than 46 genera worldwide (Cameron, 1904). Though the species in this sub-family have been collected

and recorded, enough study of their biology have not been carried out, and little is known about their efficacy as biological control agents of insect pests (Farahani *et al*., 2014; Agboyi *et al*., 2019). *C. luteum* has an orange-copper like body, with dark apical 3rd metatarsus (hind legs), and a dark coloured antenna (Fig. 11).

2.4.1 Distribution and abundance of *Coccygidium luteum* (Brullé)

Coccygidium luteum (Brullé) has been recorded in many parts of the world including; Benin, Cameroon, Congo, Democratic Republic of Congo, Ethiopia, Ghana, Guinea, Kenya, Madagascar, Mauritius Mozambique, Namibia, Niger, Nigeria, Réunion, Rodriques Island, Senegal, Seychelles, Somalia, South Africa, Tanzania, Togo, Uganda, and Yemen (Van Noort, 2019; Agboyi *et al*., 2020; Canico *et al*., 2020).

Quantitative data on *S. frugiperda* larval parasitoids surveyed in selected localities of the Eastern, Volta and Central regions of Ghana reported *Coccygidium luteum* was the highest in abundance amongst two abundant parasitoid species collected, with a relative abundance estimated at 49% in the Eastern Region and 44% in the Volta Region. The Central region was the most abundant (69%). The highest total larval parasitism rate have been observed in the Eastern, Volta region and Central region at (38.8%), (10.7%) and (5.1%), respectively. *C. luteum* caused an average of 19.3% parasitism at the species level, in the Eastern region. Somanya a locality in the Eastern region of Ghana recorded the highest parasitism rate at 75.0% (Agboyi *et al*., 2020). Natural enemies sampled from Fall armyworm in Mozambique also recorded a higher trend in abundance of *C. luteum* as in the case of Ghana. Maximum parasitism of 23.9% and relative abundance of 100

were recorded for *C. luteum* with a total parasitism by different parasitoid species at 9.5% (Canico *et al*., 2020). In other parts of Africa, the case was different with respect to the abundance of *C. luteum*. East African countries, Kenya and Tanzania recorded a fewer number of *C. luteum* with a lower Fall armyworm parasitism less than 10%, unlike the case of Ghana, where *C. luteum* was more abundant and causing up to 17% parasitism (Sisay *et al*., 2018; Agboyi *et al*., 2020).

2.4.2 Biology and life cycle of *Coccygidium* luteum (Brullé)

According to Agboyi *et al*. (2020), female *C. luteum* wasps parasitized day-old first instar larvae of *S. frugiperda* by ovipositing into the body as endo-parasitoids. The parasitoid larvae feed inside the host after hatching, thereby affecting growth of the host, which is a common phenomenon in koinobiont parasitoids. Parasitoid larvae kill the host as they emerge to complete their development from the pupae to adults outside the body of the host (Haeselbarth, 1979).

The emerged parasitoid larvae develop as white cocoon and conceal themselves during pupation. The development time of the egg to pupa stage was recorded to have lasted 8-10 days, while the duration from pupal stage to adult emergence was also 8 to 10 days (Fig. 10). The mean generation time was 16.71 ± 0.14 days (95% CI: 16.42 - 17.00). The minimum and maximum generation times were 16 and 20 days, respectively. A detailed generation time study was proposed as the research had limitations in finding as the study was designed to find the effect of leaves feeding in Fall armyworm after they have been

parasitized by *C. luteum*. There was evidence of Fall armyworm feeding decrease of 89% after *C. luteum* parasitism (Agboyi *et al*., 2020).

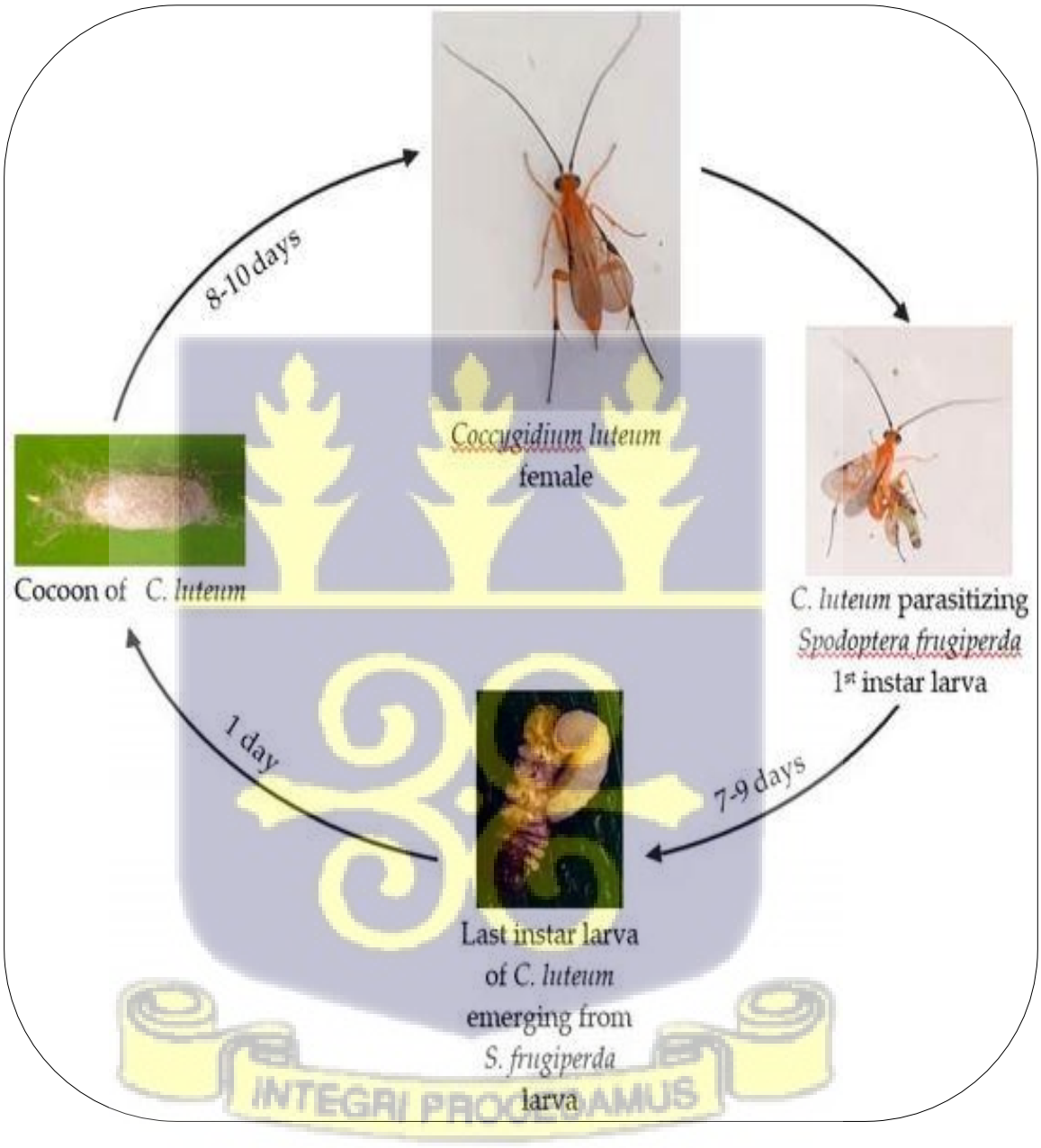


Figure 10. Generalized generation time of *Coccygidium luteum* (Source: Agboyi *et. al*., 2019).

2.4.3 Morphological identification of *Coccygidium luteum (Brullé)*

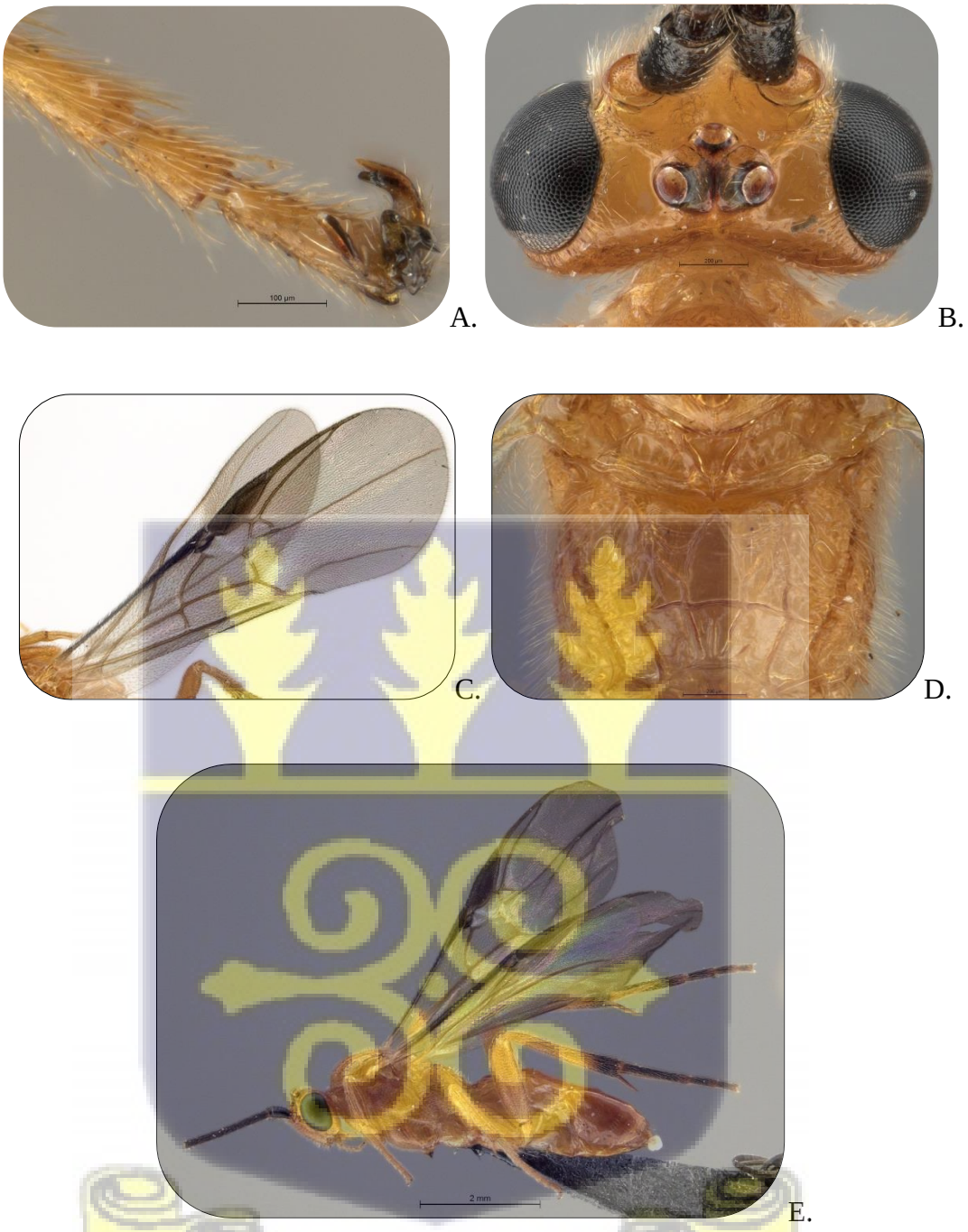


Figure 11.0: (A) claws, **(B)** Head dorsal, **(C)** Wings, **(D)** Mesosoma dorsal, **(E)** Habitus lateral of *C. luteum*. (Source: Photographs © Simon van Noort, Iziko Museums of South Africa, www.waspweb.org)

2.6 Life history strategies of parasitoids

Life history strategies are methods or plans chosen to bring about desired survival of progeny. It also involves a series of actions taken to derive effective and efficient use of resources by insect parasitoids (Godfray, 2007).

2.6.1 Ovigeny in parasitoids

The eggs laying capacity of a female parasitoid during her lifetime is dependent on interactions among three processes: the number of suitable hosts that are encountered, the number of eggs that are matured over the female life span, and behavioural manipulation of the oviposition rate (Rosenheim, 1996). Flanders (1950) classified parasitoid wasps that have all or nearly all of their eggs mature prior to the start of oviposition as 'pro-ovigenic' and those that continue to mature eggs throughout their reproductive life as 'synovigenic'. Based on various types of information used, egg maturation types have been identified (pro-ovigenic or synovigenic) in 638 species belonging to 28 families within those whose larvae parasitize other insects (the Parasitica) and the stinging forms (the Aculeata) of the Order Hymenoptera. The vast majority of these species have been found to be (98.12%, n = 626) synovigenic, while only 12 species (1.88%) are unambiguously pro-ovigenic. The average estimated number of described species in each parasitoid family, indicates that there are 27 pro-ovigenic and 611 synovigenic species which accounts for 4·23% and 95·77%, respectively (Jervis *et al*., 2001).

A continuum of ovigeny (egg production) is likely to exist, in which species can range from pro-ovigenic, where all eggs mature at emergence (Family: Platygastridae,

Aphelinidae), through weakly synovigenic, where most eggs mature at emergence (Family: Trichogrammatidae, Elasmidae), to extremely synovigenic where no eggs are mature at emergence (Jervis and Copland 1996) which typical examples are Family: Ichneumonidae and Braconidae. According to Flanders (1950), in parasitoids whose eggs mature upon emergence (pro-ovigenic) have a shorter life-span than synovigenic ones which agrees with findings from life-span test of Braconidae, where the life span of pro-ovigenic against synovigenic parasitoids proved that the life span of synovigenic species is indeed significantly greater than that of pro-ovigenic species ($P < 0{\cdot}0001$) (Jervis *et al*., 2001). Data for ovigeny indices for 67 species strongly support previous suggestions that there is a continuum of ovigeny from an index of 0 (extreme synovigeny) to an index of 1 (pro-ovigeny) (Jervis and Copland 1996; Quicke, 1997).

In the family Braconidae, ovigeny index ranged from 0 to 0.74 which indicates extreme synovigeny to weak pro-ovigeny. *Cotesia species*, *Cotesis flavipes* (Cameron) and *Cotesia plutellae* (Kurdjumov) recorded ovigeny of 1 and 0.32, respectively (Lim 1982; Potting *et al.* 1997). This implies *Cotesia species* are more likely to be pro-ovigenic with a few exhibiting weak synovigeny. Host feeding species tend on average to have a lower ovigeny index than non-host feeders and this difference was found to be significant ($P = 0{\cdot}0002$) (Jervis *et al*., 2001).

2.6.2 Host feeding in larval parasitoids

Host feeding is the consumption of host hemolymph and tissues by adult parasitoids. Flanders suggested it is confined to synovigenic species. The reasoning behind this is as

follows: whereas female pro-ovigenic and weakly synovigenic wasps presumably require nutrients more for maintenance purposes, strongly synovigenic wasps need nourishment for both maintenance and reproduction. Trehalose and proline are major metabolism fuels, both of which can occur in significant amounts in host blood and can potentially be used for somatic maintenance in the broad sense (Gilbert and Jervis 1998). Host tissues and blood are known to be a rich source of egg production nutrients for insects. Since synovigenic parasitoids have no matured eggs at emergence, they need to feed on host to acquire the necessary nourishment for egg production, which proteins are most essential with this regard.

Some synovigenic parasitoid species which engage in host-feeding can apportion substantial nutrient to reproduction, over a long time period (2 weeks in *Dinarmus basalis* Rondani), nutrients originally acquired from a single meal taken early on in life (Rivero and Casas 1999a). Although host feeding has been undeniably recorded in many synovigenic species (Jervis and Kidd 1986) it is unclear whether it is actually limited to synovigenic wasps (Jervis *et al*., 2001).

2.6.3 Egg resorption in parasitoids

The production of yolk-deficient (hydropic) eggs is associated with a higher proportion of eggs mature upon female emergence than the production of yolk-rich (anhydropic) eggs. Egg resorption capability is a unique strategy among anhydropic egg-producers, as hydropic eggs contain little or no yolk for the female to salvage. Egg resorption is also associated with a relatively low proportion of oocytes mature upon female emergence

where resorption usually precludes oviposition and can be a time-consuming process (Jervis and Kidd, 1986). Longer-lived species are also capable of egg resorption, where long life span being a trait predicted to be associated with a low proportion of oocytes mature upon emergence.

2.6.4 Parasitoid host immune counter response

Parasitoid reproductive strategy is linked to the mode of parasitism. Two life-history strategies are widely known to occur among parasitoids with respect to development mode: the first is 'koinobiosis' in which the host continues to develop, grow (if attacked as a larva), reproduce (if attacked as an adult), and remain active after attack, and secondly, 'idiobiosis' in which the host does not continue to develop, grow or remain active after attack (Godfray, 2007).

Koinobiont endoparasitoids must overcome their host defence mechanism to survive, same as *Coccygidium luteum* and all other koinobionts. In general, the defense mechanisms developed by koinobionts are passive and/or active. In the case of passive defense, females coat their eggs with a layer of protein to protect them against the host's haemocytes or lay them in organs away from the circulation of hemocytes (Schmidt *et al*., 2001; Godfray 2007). With an active defense mechanism, the endoparasitoid injects venom proteins or a virus during oviposition, in order to modify the behaviour and damage the immune system of the host. For example, another Braconidae, *Chelonus blackburni* (Cameron), is able to decrease significantly the number of haemocytes in a parasitized *Helicoverpa armigera* larva (Order: Family) and affect its midgut, thereby reducing the leaf consumption rate of the host and leading to death (Sanap *et al*., 2016).

For *C. luteum*, venom is suspected to be deployed in the parasitism mechanism, as there is a rapid reduction of feed consumption of after parasitism. The female parasitoid struggles with the host during oviposition provoke an elevation of dopamine level in the parasitized larva. Dopamine plays an important role in the retardation of larval growth (Yamanaka *et al*., 1996; Fang *et al*., 2011). The reduction of host leaf consumption is a usual phenomenon for endoparasitoid wasps (Beckage and Riddiford 1978; Beckage and Riddiford 1982).

Host defense

In insects and other arthropods, haemocyte-mediated encapsulations characteristically are accompanied by melanogenesis, a feature that has long been viewed as evidence that the process constitutes an essential component of the defense response of these animals (Bidla *et al*., 2007; Cerenius and Soderhall, 2004; Christensen and Soderhall, 2005; De Gregorio *et al*., 2002a; Ligoxygakis *et al*., 2002; Nappi and Christensen, 2005; Liu *et al*., 2007). Activated PO (PHENOLOXIDASE) have been reported to function in cuticular melanization and sclerotization, and it is generally believed that at least some of the enzymes and products of these reactions play a critical role in the defense reactions of insects against invaders, although the latter issue still remains a matter of debate (Cerenius and Soderhall, 2004; Schnitger *et al*., 2007).

Immune suppression of host by parasitoids

Immune suppression of host by insect parasitoids has mainly come from investigations of effects of parasitism or parasite-derived factors in vivo and in vitro. Diminished host

phenoloxidase activity and lack of melanotic encapsulation has been reported in parasitoid-infected Lepidoptera. Successful wasp species introduce virulence factors into the host that suppress haemocyte-mediated encapsulation (Beckage *et al.*, 1990; Strand and Noda, 1991; Beck *et al.*, 2000).

2.6.5 Spermatogeny

Few research have come out with findings that propose some parasitoid species show variability in life-history parameter in spermatogeny index for male parasitoid wasps (Boivin *et al.*, 2005). Prospermatogenic species have an index of 1, have all their spermatozoids mature at emergence and do not produce more spermatozoids later in life. At the other end of the spectrum, synspermatogenic species have no spermatozoids at emergence and produce them later in life (Wilkes, 1966). According to Roitberg *et al.* (2001), the fitness of male parasitic wasps depends on both their production of spermatozoids and their capacity to acquire mates.

One opiine braconid, *Fopius arisanus*, is synspermatogenic. Sperm migrate from the seminal vesicles to the testes several days after male emergence before mating the first time in 4 days (Quimio and Walter 2000). Most species appear to be moderately synspermatogenic; males emerge with spermatozoids and can mate shortly after emergence, but they have the capacity to produce spermatozoids throughout their life (Boivin *et al.*, 2005).

In *Bracon hebetor* (Hymenoptera: Braconidae) males do not mate until several hours after emergence, although spermatozoids are present in the seminal vesicles (Gerling and

Rotary 1974; Ode *et al*., 1996). Males produced more spermatozoids throughout their life when they have exhausted their daily supply of spermatozoids after a series of mating each day (Ode *et al*., 1996). The above phenomenon suggests that this species is also moderately synspermatogenic.

Parasitoids in the family Braconidae are known to Fall within moderately synspermatogenic (0 < index < 1) and Synspermatogenic (index = 0) (Ramadan *et al*., 1991; Quimio and Walter, 2000).

2.6.6 Interactions between parasitoids and its implication

Competition experiments data using larvae of three species of *Gonatocerus* spp. (Hymenoptera: Mymaridae), egg parasitoids of the sharpshooter *Tapajosa rubromarginata* (Hemiptera: Cicadellidae) as a case study. This study tests the influence of intrinsic interspecific competition between immature stages within on an individual host, and parasitoid arrival order among the three parasitoid species.

The results showed that the species differed in competitive behaviour; some species were better competitors than others. Individuals arriving earlier had a competitive advantage; the weaker species were able to out-compete the stronger ones if the time advantage was longer than 18 h. All the species avoided already parasitized hosts, but in different degrees (Octavio *et al*., 2018).

2.6.7 Competition effects on mortality.

Interspecific competition among parasitoids might have a devastating effect on the population dynamics of insects (Isenhour, 1988; Xu *et al*., 2013). Competitive interactions for resources can occur between parasitoid species exploiting the same host either as extrinsic competition between adults exterior the host substrate or as intrinsic competition between parasitoids developing within the same host (Cusumano *et al*., 2012). Both intrinsic and extrinsic interspecific competition mechanisms are known to exist amongst Braconidae wasps, parasitizing same host species (Paranhos *et al.*, 2013; Cancino *et al.*, 2014; Murillo *et al*., 2016). Competition between species in a natural enemy guild initiates coexistence between species in the same niche (Feng *et al*., 2015). This phenomenon may cause partial or complete displacement (Reitz and Trumble, 2002).

According to Jeffries and Jeffries and Lawton (1984), competition between different parasitoid species may usually lead to species niche separation, as niche partition is not only an effect of resource-based competition. Competitive interactions between species usually occur in spatial and temporal co-occurrence, where the niches overlap (Kaplan and Denno, 2007).

A larger abiotic and biotic environmental complexity might allow for separate habitats and enhance coexistence in an area (Costamagna and Landis, 2004). The success of released parasitoids in biological control programmes is impaired by competition amongst parasitoids (Wang *et al.*, 2008; Cabello *et al.*, 2011). It is therefore important to undertake an impact evaluation of the exotic species on the native ecosystem before the release (Alyokhin and Messing, 2003).

Previous experimental studies of compensation by resource populations in response to mortality have considered direct mortality or lethal effect of natural enemies through manipulating enemy populations (Huffaker and Matsumoto 1982; Graham and Lambin 2002; Lane and Mills 2003) or artificially imposed stage-dependent mortality (Cameron and Benton 2004).

Koinobiont parasitoids are example of such a natural enemy. They inject their hosts with an egg or several eggs, where upon hatching the young parasitoid allows the host to live until some later stage (Haeselbarth, 1979). The duration of delay in host death is often determined by the age or size of the host when it is parasitised and also by the cue from the host to the juvenile parasitoid, signaling that the host contains enough resources for the parasitoid to complete its development (Godfray, 1994). Attack by parasitoids with this life history strategy generates a mixed host population that comprises both not parasitized healthy and parasitized hosts. This phenomenon leads to competitive inter-actions between the parasitoids. Any changes in behaviour, physiology or growth of the parasitized host before the initiation of destructive feeding by the parasitoid larvae have a sub lethal effect on the host, which are better known and encountered in host/pathogen interactions (Sait *et al*. 1997; Boots and Norman 2000).

2.6.8 Competition effects on parasitism and the survival of healthy host larvae.

Adult female parasitoid introduced earlier to the host, usually cause a longer period of competition between parasitized and healthy larvae. Parasitoid introduction on a later day

leads to shorter periods of competition with parasitized larvae and have no significant reduction in the survival of healthy larvae (Cameron *et al*., 2005).

2.6.9 Host species effects on the survival and clutch size of parasitoids (Braconidae).

In a study that investigated the correlation between host suitability and earlier studied host-finding behaviour of two closely related braconid larval parasitoid species, the generalist *Cotesia glomerata* (L.) and the specialist *C. rubecula* (Marshall) (Hymenoptera: Braconidae). the capability of both parasitoid species to parasitize and develop in three *Pieris* host species was compared, thus, *P. brassicae* (L.), *P. rapae* (L.) and *P. napi* (L.) (Lepidoptera: Pieridae). In laboratory experiments, the effect of host species on fitness parameters such as survival, development, sex ratio and size of parasitoid progeny was measured. The results show that *C. glomerata* is capable of developing in the three host species, with significant differences in parasitoid survival, clutch size and adult weight among *Pieris* species. The host range for development was more restricted for *C. rubecula*. Although *C. rubecula* is physiologically able to develop in *P. brassicae* larvae, parasitoid fitness is negatively affected by the host species compared to its most regular host, *P. rapae* (Brodeur *et al*., 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Insects collection and rearing

The study was conducted at the Plant Protection and Regulatory Services Directorate (PPRSD) and Centre for Agriculture and Bioscience International (CABI) biological pest control laboratory in Ghana. Sampling was done to collect two parasitoids specimens in maize field in Eastern region of Ghana. Young Fall armyworm larvae (larval instar 1-3) were collected (Fig. 12) and separated one each into a plastic aerated sauce cups (650 mL volume) and kept under 26± 1 ºC temperature and 60 ± 5 HR relative humidity until the emergence of parasitoid adults or *S. frugiperda* moths at the laboratory. Adult parasitoids and *S. frugiperda* that emerged was reared in separate aerated plastic cages measuring 18 dm^3 and 50 dm^3, respectively. Both insects Adults were fed with 10% honey solution soaked in cotton wool daily. Complete Randomized Design (CRD) was used for the laboratory studies.

Figure 12.0: Fall armyworm sampling. A = Collection in the field. B = larvae and feeding material in a collection bowl.

3.2 Laboratory study

The field-collected Fall armyworm larvae samples were transported to the PPRSD laboratory for assessment of the likelihood of parasitism and parasitoid emergence (Fig. 13). The larvae were checked on daily basis, taking records of date of happenings until parasitoid and/or moth emergence. The *C. luteum* and *C. icipe* adults that emerged were used in the test to estimate the life span, behaviour, interactions with each other, and potential to control the Fall armyworm.

A. B.

Figure 13. Emerged parasitoid adult in aerated (18 dm^3) sauce cup = A, Parasitoid colony in a netted plastic container = B.

Fall armyworm rearing

Maize was planted at two weeks intervals in a screen house to feed Fall armyworm larvae (Fig. 14). Ten (10) Fall armyworm larvae were kept in an aerated plastic bowl with tissue

paper and maize leaves which were changed daily (Fig. 15). This served as the colony that provided the Fall armyworm host for the tests (Figs. 16-18).

A.   B.

C. 

Figure 14. From field to laboratory. A = Maize in screen house, B = fresh maize collected for FAW feeding, C = FAW Pupae collected from rearing.

Figure 15. FAW and parasitoid rearing setup.

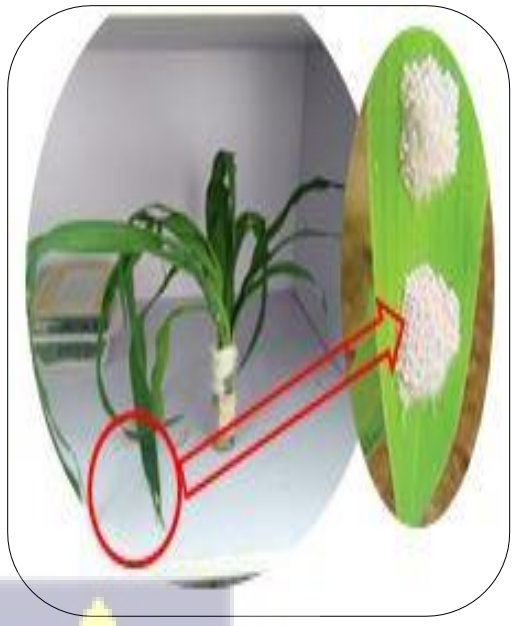


Figure 16. FAW egg mass on young maize plant.

A.

Figure 17. FAW female moth.

B.

Figure 18. Adult in cage with plant and 10% honey soaked cotton.

3.3 Identification of the Fall armyworm larval instar preferred by *C. luteum* and *C. icipe*

- **Choice test**

Choice tests were conducted by exposing various instars of Fall armyworm to one mated female *C. luteum* and *C. icipe* (Figs. 19-20).

One (1) freshly emerged, 1-day-old, 2-day-old, 3-day-old, 4-day-old, 5-day-old, 6-day-old, 7-day-old, 8-day-old and 9-day-old Fall armyworm larvae were introduced to one mated female of each parasitoid in a petri dish (diameter: 8.5cm; height 1.3cm; volume 60 ml) at the same time, making a total of 10 larvae in a petri dish with a single female parasitoid species at a time. The Fall armyworm larvae were collected after 30 minutes and individually separately in aerated sauce cups (18 dm^3 volume) to prevent cannibalism. The time for each female parasitoid on Fall armyworm larva was recorded. The parasitized Fall armyworm larvae were observed daily to record emergence of a parasitoids or continual development of the Fall armyworm. The test was replicated five times. Preference was assessed by the highest number of Fall armyworm larva parasitized at each instar (age in days) and comparing the time each female parasitoid used to parasitize the larvae.

- **Non-choice test**

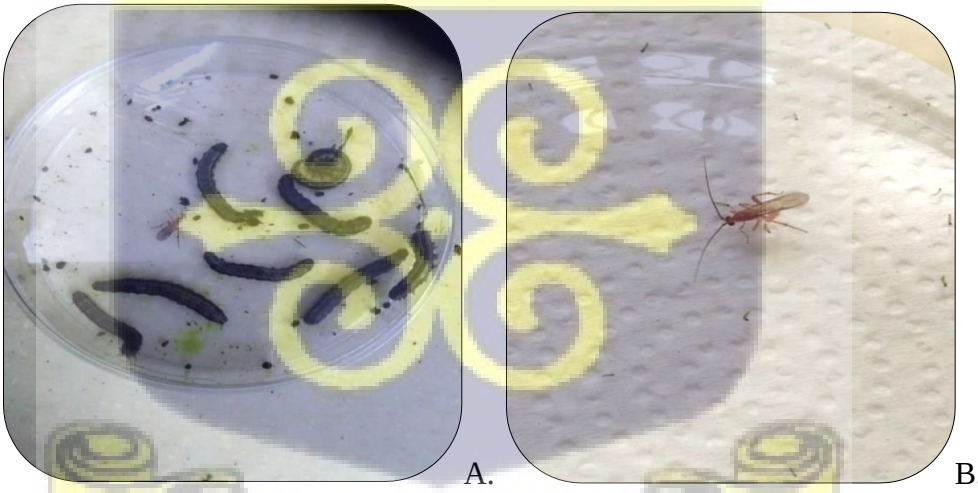
Non-choice test was conducted by separately exposing 10 newly emerged Fall armyworm larvae individually to *C. luteum* and *C. icipe* females in a petri dish (diameter: 8.5cm; height 1.3cm; volume 60 ml) to assess the capability of the parasitoids to reproduce. Same was done by exposing 1-day-old to 9-day-old to the parasitoids. Five (5)

replications were done for the test. A control was set up with un-parasitized larvae to compare the mortality of parasitized and un-parasitized larvae. Larvae were considered parasitized only when a parasitoid wasp emerged from the host. Ideal Fall armyworm preference was calculated as a function of percentage parasitism, adult parasitoid emergence and Fall armyworm mortality after parasitism, using the equation below. The ideal preferred larva had the highest ideal preference value.

EQUATION

IDEAL PREFERENCE =

$$\frac{(\ \%\ \text{Parasitism}\ (\mathbf{PA})\ -\ \%\ \text{Mortality}\ (\mathbf{M})\ \ )}{(\ \%\ \textit{Parasitism}\ (\boldsymbol{PA})\ +\ \%\ \textit{Adult parasitoid emeregence}\ (\mathbf{PE})\ )}$$



A. B.

Figure 19. *C. luteum* in petri dish with 9-day-old FAW larvae **(A),** and newly emerged FAW larvae with *C. luteum* **(B)** in a Non-choice test.

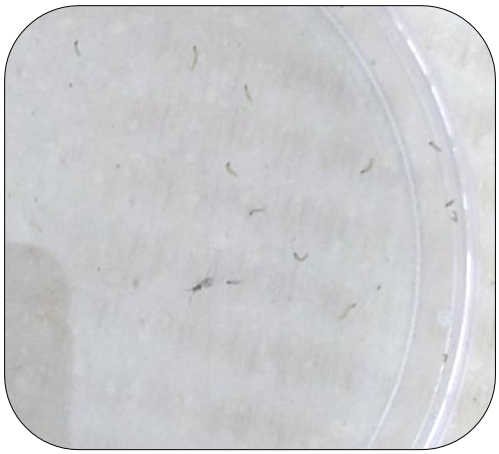
A.   B.

Figure 20. *C. icipe* in petri dish with newly emerged FAW **(A)**; *C. luteum* with different FAW larvae in a choice test **(B)**.

3.4 Assessment of the parasitism potential of parasitism of *C. luteum* and *C. icipe*

One mated female of each parasitoid was introduced into a petri dish (diameter: 8.5cm; height 1.3cm; volume 60 ml) after 12 hours of emergence. A total of fifty (50) 3-day-old and 1-day-old preferred larval instars were exposed to each female *C. luteum* and *C. icipe*, respectively on daily basis for six days (Figs. 21-22). The exposed larvae were removed from the petri dish with female after 2 hours and kept separately in aerated cups each day. The female parasitoids were kept with a male parasitoid together in a cage (60 ml volume). The larvae were fed with tender maize leaves until the emergence of parasitoid larvae or Fall armyworm pupa. The parasitism capacity of each parasitoid species was calculated by cumulating the total number of parasitized larvae, out of the total larvae exposed to parasitoids females. The same number of Fall armyworm larvae was kept as control. The tests were conducted in the laboratory at 26 ± 1 ºC and 60 ± 5 HR ,with 5 replications.


A.


B.

Figure 21. Separation of Fall armyworm larvae into sauce cups with tender leaves **(A),** Sauce cups covered **(B)**.

Figure 22. Capacity test set up with mated female parasitoids in petri dishes and cages holding adult male parasitoids for each replicate.

3.5 Life span of *C. luteum* and *C. icipe*

Fall armyworm 3days old and 1day old larva was exposed to a mated female of *C. luteum* and *C. icipe*, respectively in separate cages for 1 hour to be parasitized. After recording the day and time the Fall armyworm larvae were parasitized, a total of 50 individuals of each of *C. luteum* and *C. icipe* parasitized larvae were kept in aerated transparent cup (18 dm^3 volume) for the life span study. The parasitized larvae were given tender maize leaves, until the emergence of the last larval instars of each parasitoid and the formation of the parasitoid pupae. The date and time of the pupae formation were recorded and the parasitoid pupae followed until the emergence of the wasps.

A cohort of fifteen (15) females and 15 males of *C. luteum* and *C. icipe* freshly emerged were kept together in aerated cage containing an artificial diet (sterilized cotton wool soaked into 10% honey solution) which was changed daily. Date of emergence of the parasitoid adults used for the test was recorded. The number of dead wasps was recorded every 24 hours, as well as the sex. Dead male and female *C icipe* were separated with the help of a microscope due to their smaller size (Fig. 23). A total of five replications were done during the tests.

The time (days) in completing lifecycle (life cycle duration) was obtained by calculating the interval between the day of egg laying (parasitism) until death of adult parasitoid. The tests were conducted in laboratory, under 26 ± 1 ºC and 60 ± 5 HR.

Voltinism (number of generations per year) was calculated with the average generation time. Voltinism = average generation time / 12 (number of months in a year).

Figure 23. Separating male and female *C. icipe* under microscope.

3.6 Interaction (synergy/competition) between *C. luteum* and *C. icipe* in the presence of Fall armyworm larvae

The interaction test was conducted in laboratory in two steps as followed:

- **Step 1: Concurrent parasitoid introduction to Fall armyworm**

A male and female of *C. luteum* and *C. icipe* were kept together in an oviposition cage (60 ml), and provided with an artificial diet (cotton wool soaked into 10% honey solution). After 24 hours in the cage, 30 of 2days old Fall armyworm larvae were exposed to female *C. luteum* and *C. icipe* at a time for 30 minutes in a Petri dish (diameter: 8.5cm; height 1.3cm; volume 60ml). The Fall armyworm larvae were removed from the petri dish and kept separately in aerated sauce cups (18 dm^3 volume) and provided with tender maize leaves, until the emergence of the parasitoids. Five replications were done for this test.

- **Step 2: Sequential parasitoid introduction to Fall armyworm to parasitoids**

Thirty (30) of 2 days old Fall armyworm larvae were exposed to a 24 hour mated female *C. icipe* for oviposition in 2hour period in a petri dish (diameter: 8.5cm; height 1.3cm; volume 60ml) (Fig. 24). The parasitized larvae was immediately removed after the 2hour duration and exposed to a 24 hour mated female *C. luteum* to observe if the latter will accept parasitizing them again. Larvae accepted and parasitized by *C. luteum* female after initial *C. icipe* parasitism will be removed from the oviposition cage for the second time and kept separately in aerated sauce cups (18 dm^3 volume) and the time for parasitism recorded. The Fall armyworm larvae were given tender maize leaves until the emergence of parasitoid larvae. The parasitoid species that emerged were recorded and used to assess the multi-parasitism potential of *C. luteum* (Fig. 24).

Similar test was conducted by making the Fall armyworm larvae being parasitized firstly by *C. luteum* and secondly by *C. icipe*, in order to assess also the multi-parasitism potential of *C. icipe*. Five (5) replications were done for this test.

Figure 24. Interaction test set up with *C. luteum* introduced **(A)**: *C. icipe* **(B)**.

3.7 Data Analysis

Life span and generation time of parasitoids were analyzed with descriptive statistics. Difference within parasitism levels, mortality and adult parasitoid emergence in preference, parasitism potential and competition test was analyzed with one-way analysis of variance (ONE-WAY ANOVA), using Turkey mean separation test to separate means. Fisher Pairwise LSD comparism was use for preference choice test mean separation. Two-Sample *t*-test was used in comparing total parasitism potentials of *C. luteum* and *C. icipe*. All statistical analysis was run with Minitab 17® statistical software at 95 % confidence interval (Minitab v.17, Minitab, State College, PA, USA).

CHAPTER FOUR

4.0 RESULTS

4.1 Parasitoid life span

4.1.1 *Cotesia icipe*

Cotesia icipe parasitoid life span was analyzed with a descriptive statistics on 250 total sample size. The duration for *Cotesia icipe* oviposition was between 12 hours to 1 day (24 hrs.) (Fig. 4.1.3A; Fig. 4.3A). Egg to larva duration ranged from 7 to 8 day and 6.85 ±0.06 days on average. The period from larval emergence to pupa was 4 to 8 hours, averaging at 3.77 ±0.09 hours. Duration of development from the pupa stage to adult emergence ranged from 3 to 4 days with an average of 2.97 ±0.03 days. Duration of egg to adult emergence was 10 to 11 days and 9.82 ±0.09 days on average. Total adult life span ranged from 7 to 15 days and 7.17 ±0.26 days on average. The total generation time was 17 to 25 days and 16.99 ±0.29 on average (Fig. 4.1.7B; Fig. 4.1.9). The total number of generations was between 15 to 21 generations in a year (multivoltine) (Table 4.1.1; Fig. 4.1.1). There was significant difference between male and female adult life span with means of 7.14 and 8.34 days, respectively (P = 0.006) with 1.2 days estimate for difference in favour of females (Fig. 4.1.4).

Table 4.1.1: Descriptive Statistics of *Cotesia icipe* life span.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum
EGG TO LARVA	250	0	6.8480	0.0647	1.0223	7.0000	8.0000
PUPA TO ADULT	250	0	2.9720	0.0323	0.5102	3.0000	4.0000
ADULT LIFE SPAN	250	0	7.168	0.258	4.075	7.000	15.000
GENERATION TIME	250	0	16.988	0.293	4.625	17.000	25.000
EGG TO ADULT	250	0	9.8200	0.0904	1.4296	10.0000	11.0000
LARVA TO PUPA (hrs.)	250	0	3.7680	0.0921	1.4569	4.0000	8.0000
MALE ADULT LIFE SPAN	150	0	7.140	0.286	3.501	7.000	15.000
FEMALE ADULTLIFE SPAN	150	0	8.340	0.326	3.995	9.000	15.000

Voltinism = 15 to 21 generations in a year (multivoltine).

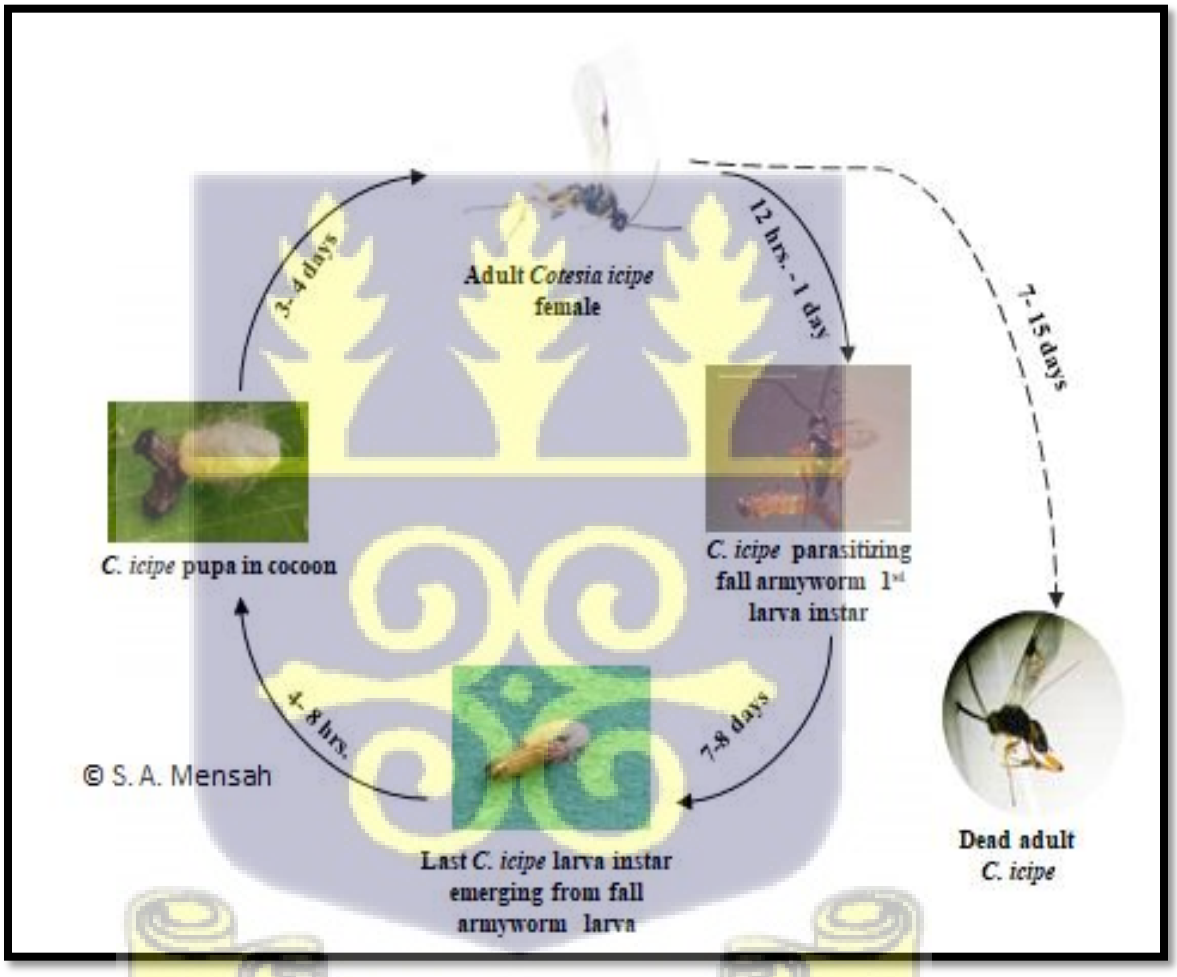


Figure 4.1.1 Annotated generation time diagram of *Cotesia icipe*.

4.1.2 *Coccygidium luteum*

Coccygidium luteum parasitoid life span was analyzed with a descriptive statistics on 250 total sample size. The duration for *C. luteum* oviposition was ≥ 1 day (24 hrs.) **(**Fig. 4.1.3B; Fig. 4.3A**)**. Egg to larva duration ranged from 7 to 10 day and 9.02 ±0.05 days on average. The period from larval emergence to pupal was 8 to 13 hours, with an average of 8.10 ±0.10 hours. Duration of development from the pupal stage to adult emergence ranged from 8 to 11 days with an average of 9.70 ±0.04 days. Duration of egg to adult emergence was 16 to 21 days and 18.70 ±0.07 days on average. Total adult life span ranged from 12 to 23 days and 11.50 ±0.35days on average. The total generation time was 30 to 42 days and 30.1 ±0.39 days on average (Fig. 4.1.7A; Fig. 4.1.8). The total number of generations was (voltinism) between 8 to 12 generations in a year (multivoltine) (Table 4.1.2; Fig. 4.1.2). There was no significant difference between male and female adult life span with means of 10.89 ±0.42 and 10.73 ±0.38 days (Fig. 4.1.5), respectively (P = 0.787) with 0.15 days estimate for difference in favour of males.

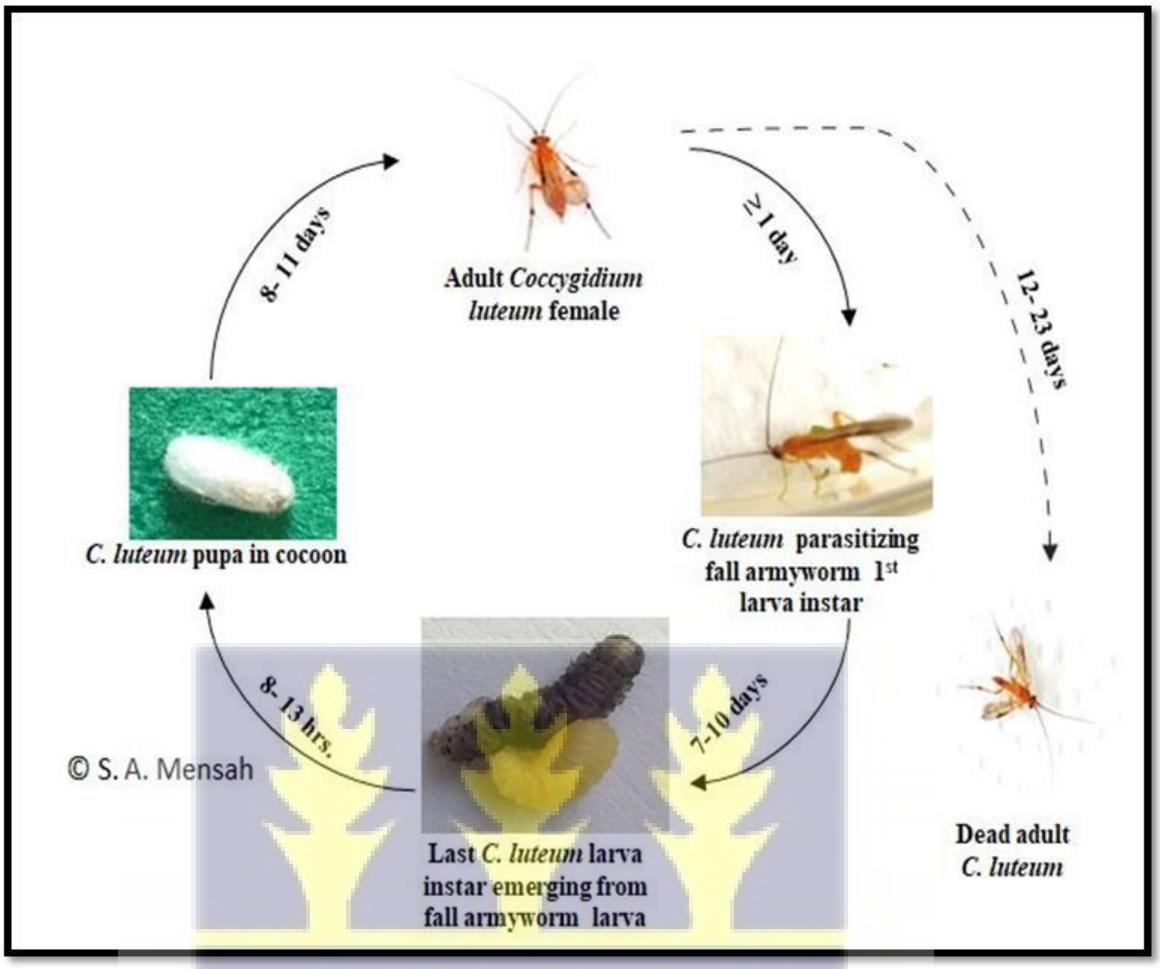
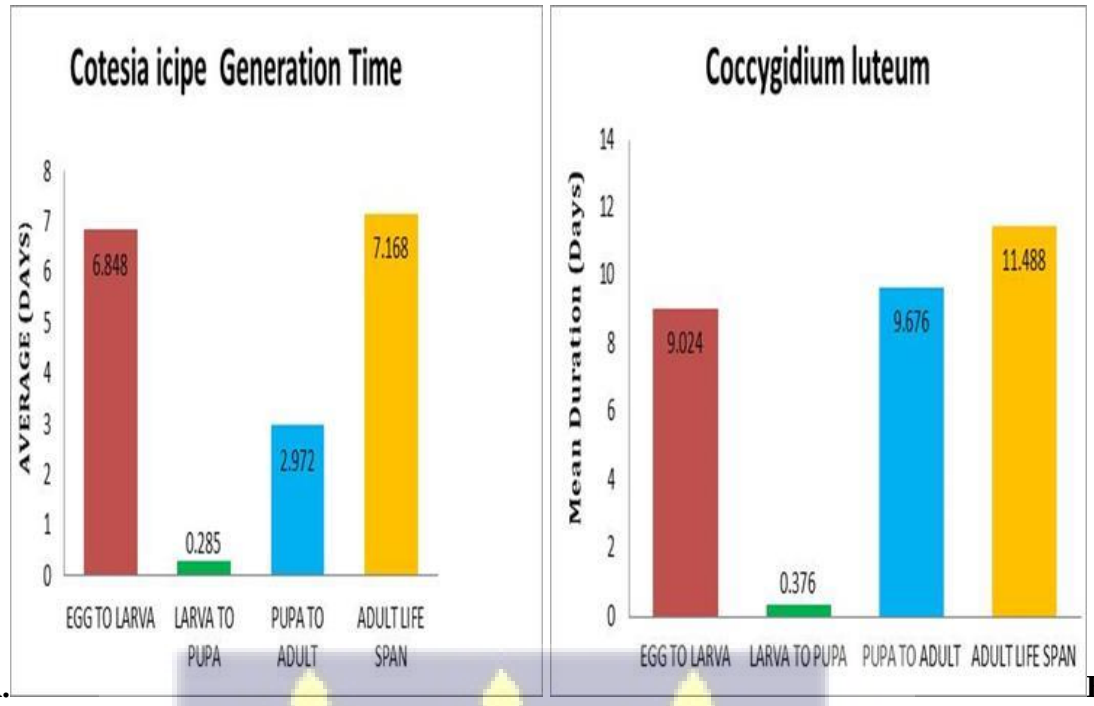


Figure 4.1.2 Annotated generation time diagram of *Cocccygidium luteum*.



A. **B.**

Figure 4.1.3: Average duration of *C. icipe* (A) and *C. luteum* (B) life stages.

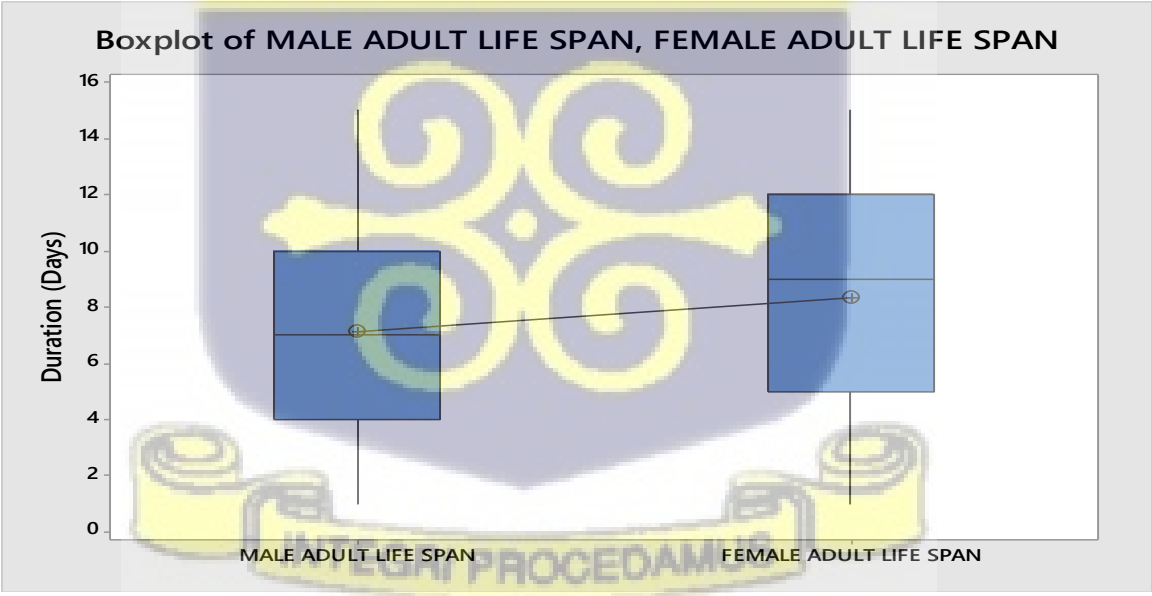


Figure 4.1.4 Boxplot of *C. icipe* male and female adult life span showing age distribution.

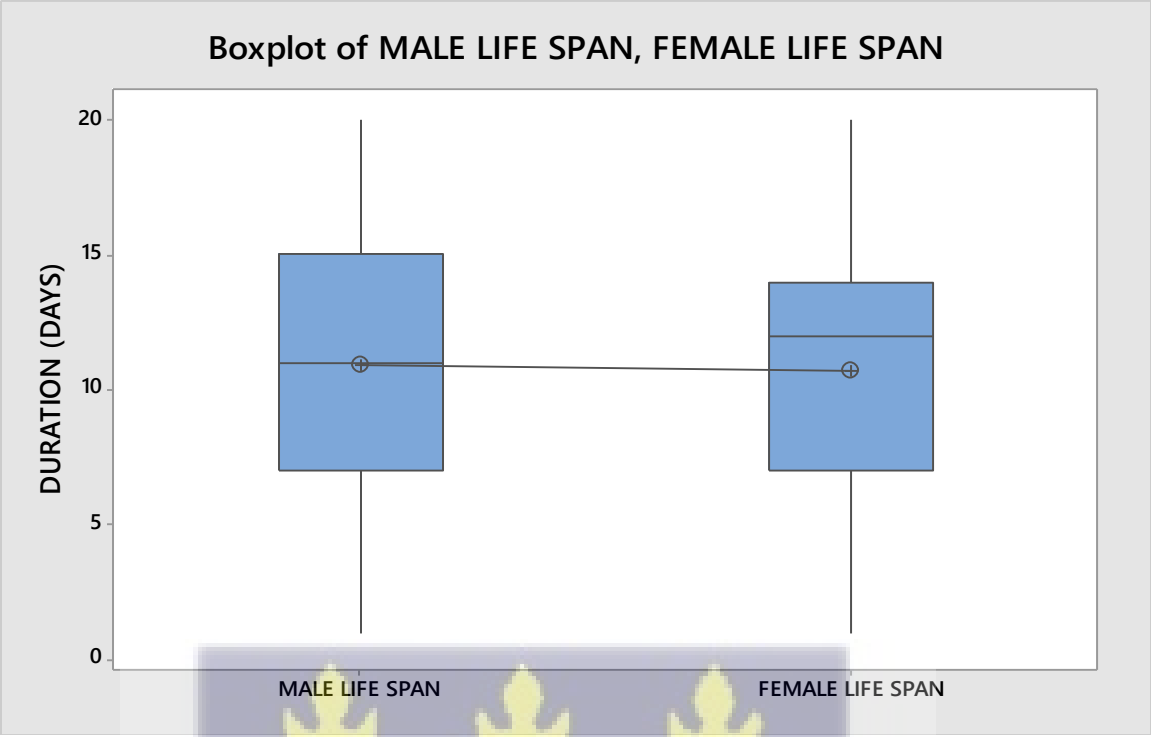
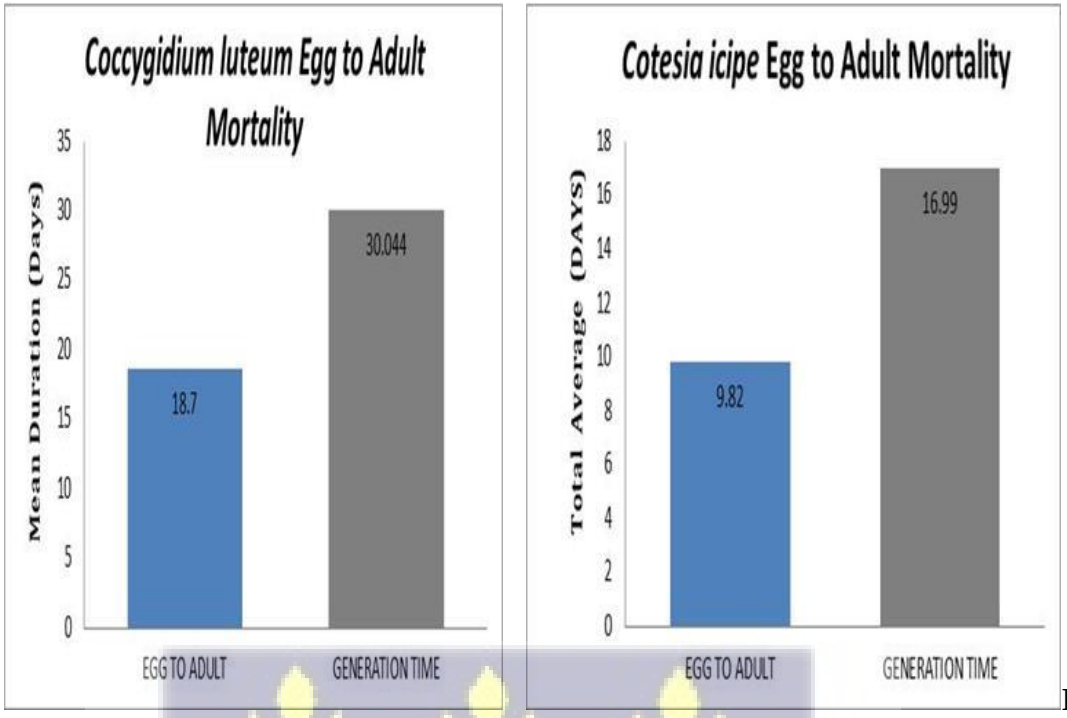


Figure 4.1.5 Boxplot of *C. luteum* male and female adult life span showing age distribution.

A. **B.**

Figure 4.1.6: *Cotesia icipe* larva with eyes and pseudo-legs before cocoon formation (A), and *Coccygidium luteum* larva emerged and form cocoon without eye and pseudo-legs (B).



A. **B.**

Figure 4.1.7: Average generation time of *C. luteum* (A) and *C. icipe* (B).

4.1.3 *Coccygidium luteum* against *Cotesia icipe* generation time

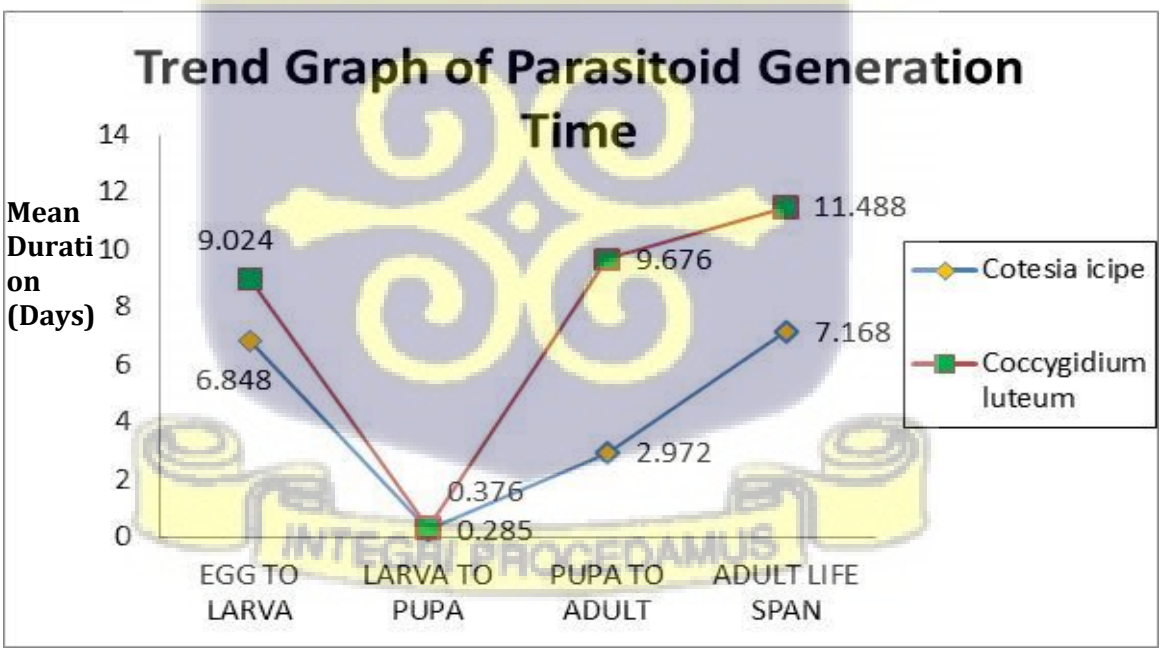


Figure 4.1.8 Trend graph of the duration of *C. luteum* and *C. icipe* life span.

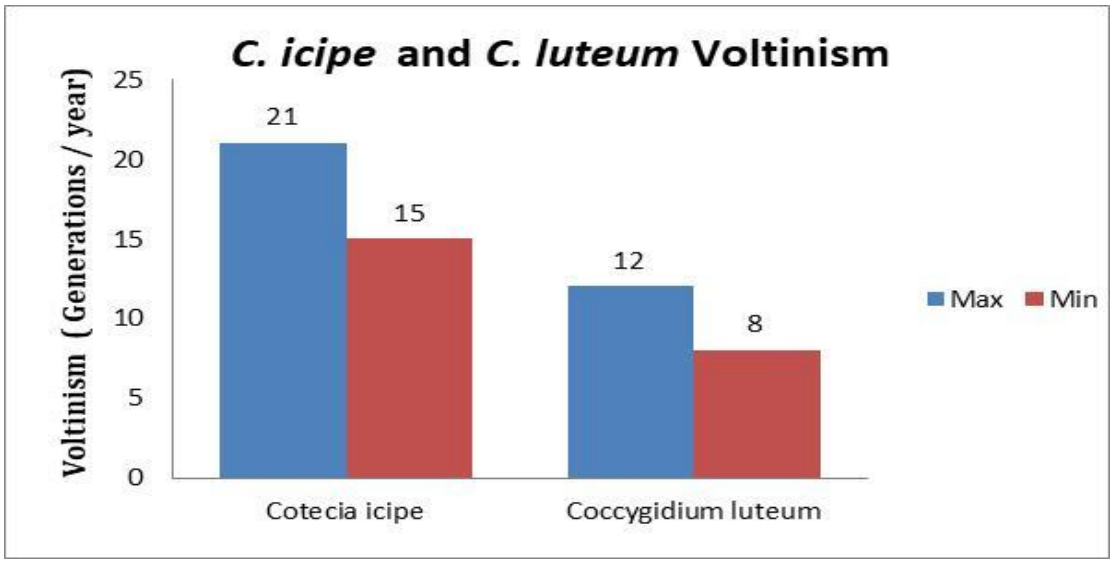


Figure 4.1.9: Maximum and minimum no. of generation per year for *C. icipe* and *C. luteum*.

Table 4.1.2: Descriptive Statistics of *Coccygidium luteum* life span.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum
EGG TO LARVA	250	0	9.0240	0.0471	0.7441	9.0000	10.0000
PUPA TO ADULT	250	0	9.6760	0.0437	0.6906	10.0000	11.0000
ADULT LIFE SPAN	250	0	11.488	0.347	5.487	12.000	23.000
GENERATION TIME	250	0	30.044	0.386	6.104	30.000	42.000
EGG TO ADULT EMERGENCE	250	0	18.700	0.0676	1.069	19.000	21.000
LARVA TO PUPA (hrs.)	250	0	8.112	0.104	1.639	8.000	13.000
MALE ADULT LIFE SPAN	150	0	10.887	0.422	5.168	11.000	20.000
FEMALE ADULT LIFE SPAN	150	0	10.733	0.379	4.644	12.000	20.000

Voltinism = 8 to 12 generations in a year (multivoltine).

4.2 Parasitoid Fall armyworm larva Preference

4.2.1. No-choice test

Coccygidium luteum

Fall armyworm larval age (days) preference of *C. luteum* was obtained by taking the % parasitism of on all ten (10) larval ages. *C. luteum* recorded an average % parasitism of 62, 74, 82, 92, 98, 98, 86, 22, 2, and 0 % out of 50 newly emerged (day 0), 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days and 9 days old Fall armyworm larvae, respectively with significant difference between groups ($P < 0.001$) (Fig. 4.2.1A). Percentage (%) adult parasitoid emergence out of larval parasitoids for newly emerged (day 0) to 9 days old Fall armyworm larva was 90.5, 87.2, 95.3, 97.8, 96.0, 100, 100, 100, 20, and 0.0 %, respectively with significant difference between groups ($P < 0.001$) (Fig. 4.2.2A). Percentage (%) mortality in newly emerged larvae to 9 days old Fall armyworm was 38, 26, 16, 8, 2, 2, 0, 0, 0, and 0%, respectively ($P < 0.001$) (Fig. 4.2.3A). Ideal larval stage preference values for newly emerged (0) to 9 days old Fall armyworm was 0.16, 0.30, 0.37, 0.44, 0.50, 0.49, 0.46, 0.18, 0.09, and 0.00 respectively. Three (3) days to six (6) days old Fall armyworm larva proved to be ideal amongst the entire group of ten different Fall armyworm age groups (day), with 4 days old larva as the most ideal for parasitism (Table 4.2; Fig. 4.2 A).

Table 4.2: Preference values for *Coccygidium luteum* no choice test.

AGE OF FAW LARVA (days)	% PARASITISM (PA)	% ADULT PARASITOID EMERGENCE (PE)	% FAW MOTALITY (M)	(PA- M)	(PA+ PE)	IDEAL FAW PREFERED (PA-M / PA+ PE)
0	62	90.5	38	24	152.476	0.16
1	74	87.1	26	48	161.143	0.30
2	82	95.3	16	66	177.278	0.37
3	92	97.8	8	84	189.778	0.44
4	98	96.0	2	96	194.000	0.50
5	98	100.0	2	96	198.000	0.49
6	86	100.0	0	86	186.000	0.46
7	22	100.0	0	22	122.000	0.18
8	2	20.0	0	2	22.000	0.09
9	0	0.0	0	0	0.000	-----

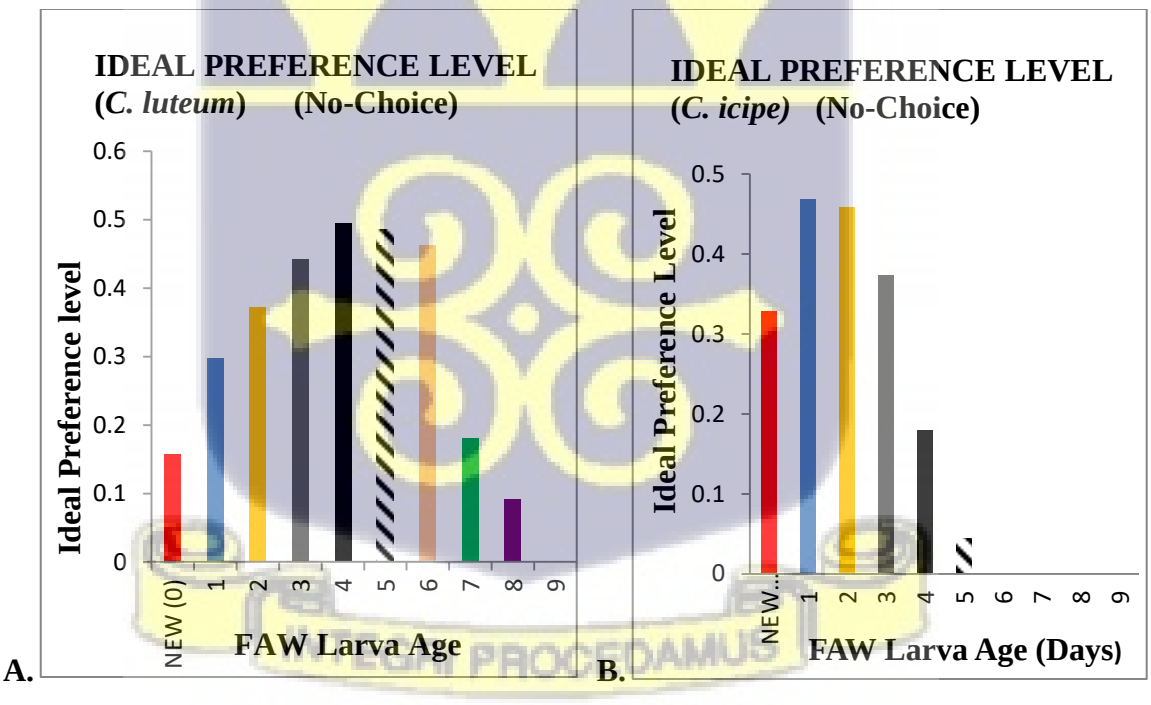
Cotesia icipe

Cotesia icipe recorded an average % parasitism of 76, 94, 92, 58, 20, 6, 0, 0, 0, and 0 % out of a total 50 newly emerged (day 0), 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days and 9 days old Fall armyworm larvae, respectively with significant difference between groups ($P < 0.001$) (Fig. 4.2.1B). Percentage (%) adult parasitoid emergence out of larval parasitoids for newly emerged (day 0) to 9 days old larva was 82.7, 93.8, 100.0, 97.2, 80.0, 40.0, 0.0, 0.0, 0.0, and 0.0 %, respectively with significant difference between groups ($P < 0.001$) (Fig. 4.2.2B). Percentage (%) mortality in newly emerged Fall armyworm larvae up to 9 days old was 24.0, 6.0, 4.0, 0.0, 2.0, 4.0, 4.0, 4.0, 2.0, and 2.0 %, respectively ($P < 0.001$) (Fig. 4.2.3B). Ideal Fall armyworm larval stage (days) preference values for newly emerged (0) to 9 days old was 0.33, 0.47, 0.46, 0.37, 0.18, 0.04, 0.00, 0.00, 0.00, and 0.00, respectively. Newly emerged (0) days to three (3) days old Fall armyworm larva proved to be preferred amongst the entire group of ten

different Fall armyworm age groups (day), with one (1) day old larva as the most ideal for parasitism (Table 4.2.1; Fig. 4.2 B**)**.

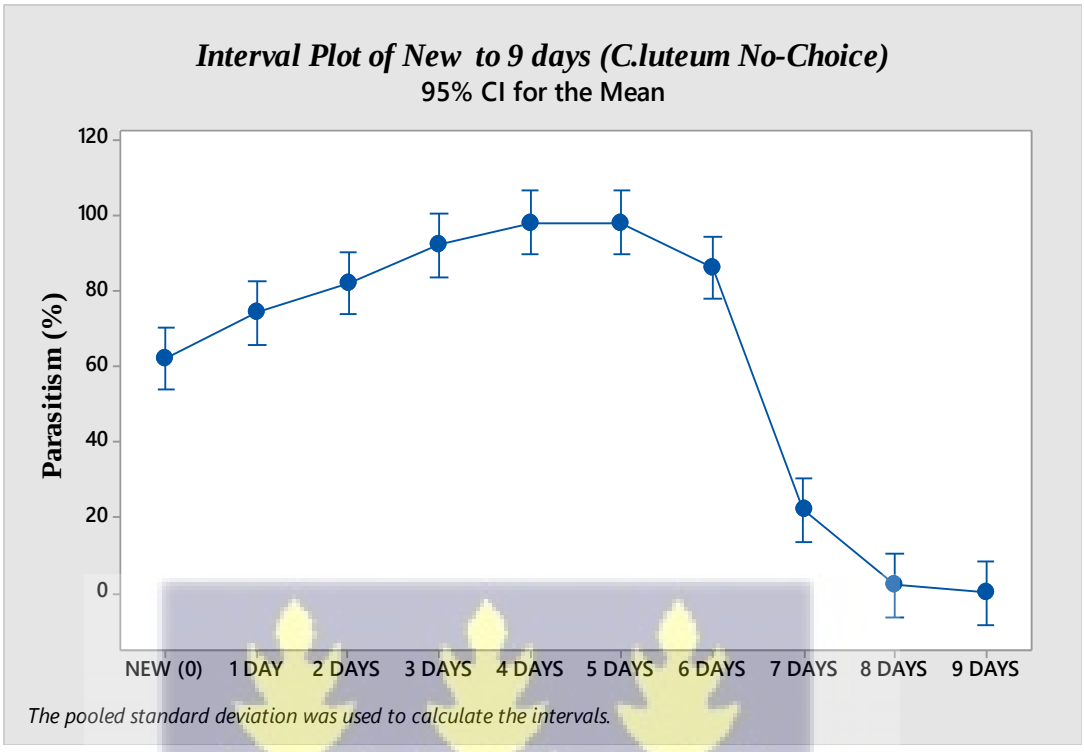
Table 4.2.1: Preference values for *Cotesia icipe* no choice test

AGE OF FAW LARVA (days)	% PARASITISM (PA)	% ADULT PARASITOID EMERGENCE (PE)	% FAW MOTALITY (M)	(PA- M)	(PA+ PE)	IDEAL FAW PREFERED (PA-M / PA+ PE)
0	76	82.7	24.0	52	158.70	0.33
1	94	93.8	6.0	88	187.78	0.47
2	92	100.0	4.0	88	192.00	0.46
3	58	97.2	0.0	58	155.18	0.37
4	20	80.0	2.0	18	100.00	0.18
5	6	40.0	4.0	2	46.00	0.04
6	0	0.0	4.0	-4	0.00	-----
7	0	0.0	4.0	-4	0.00	-----
8	0	0.0	2.0	-2	0.00	-----
9	0	0.0	2.0	0	0.00	-----

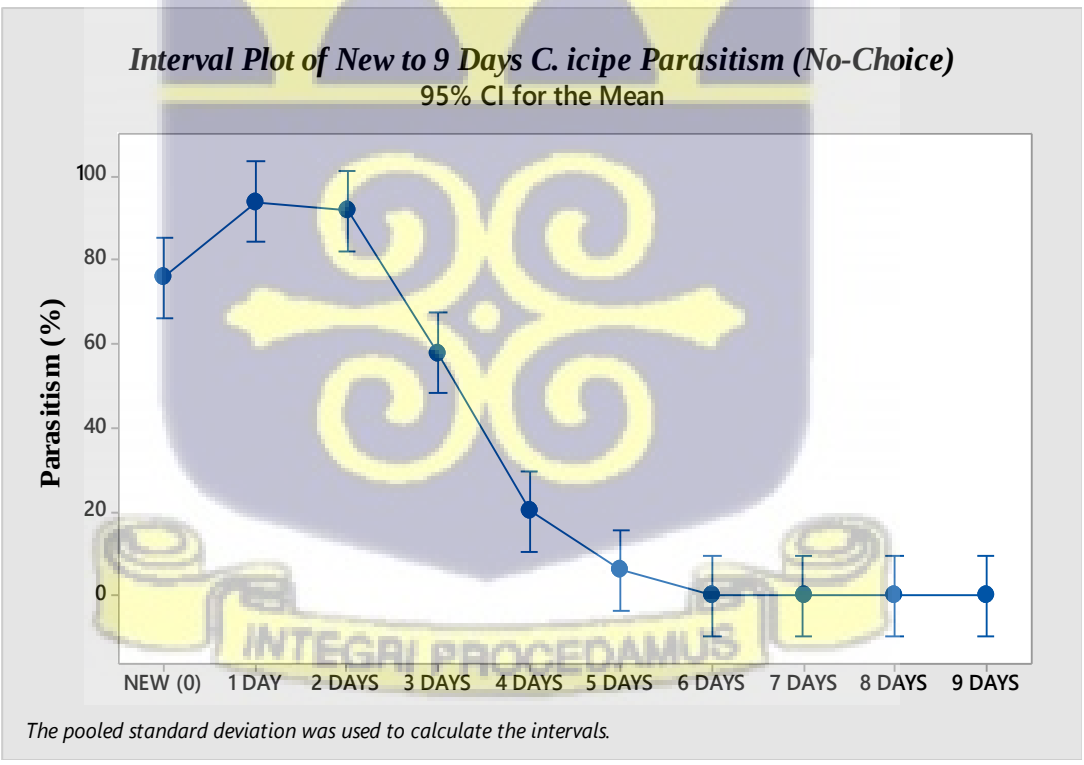


A. **B.**

Figure 4.2: Ideal Fall armyworm larval preference in *C. luteum* (A), and *C. icipe* (B) in no-choice preference test.

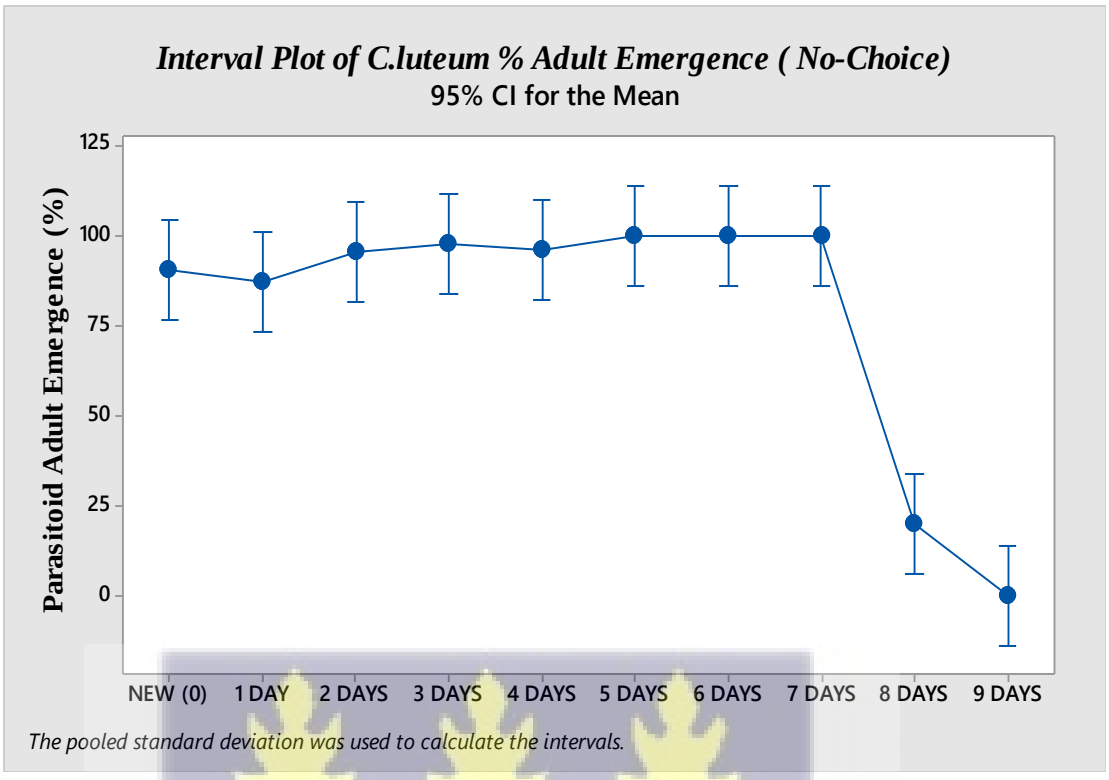


A.

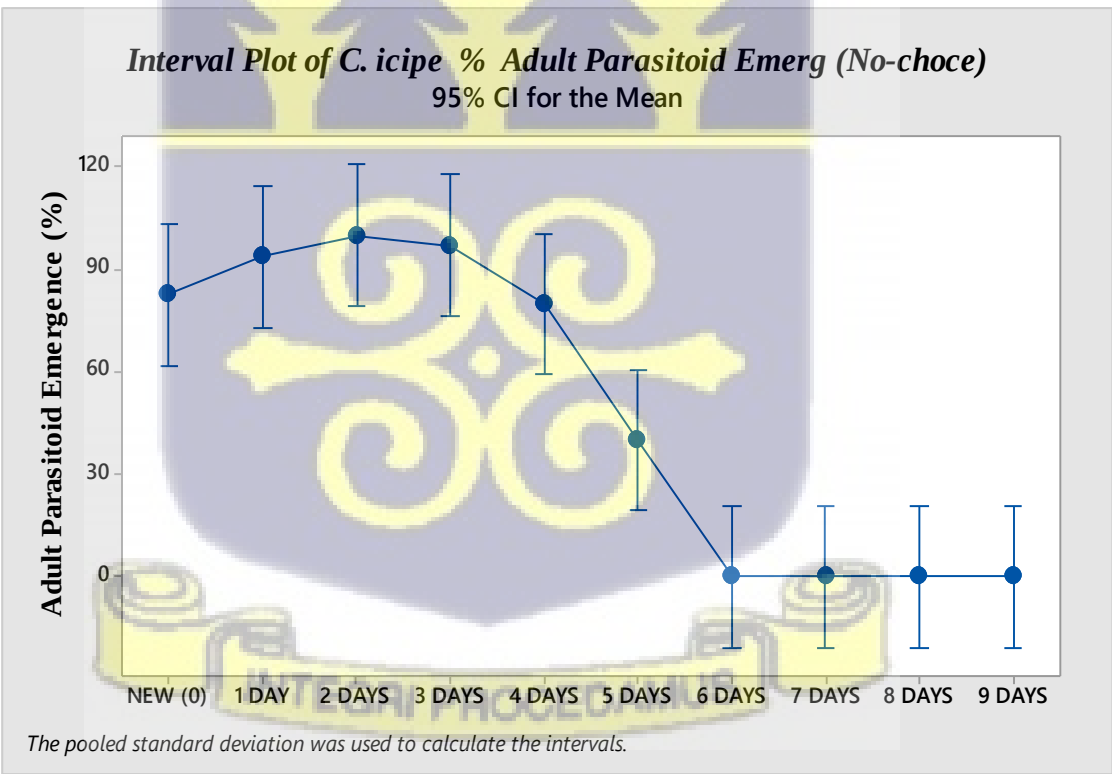


B.

Figure 4.2.1: parasitism levels of *C. luteum* (A) and *C. icipe* (B) on different Fall armyworm stages.



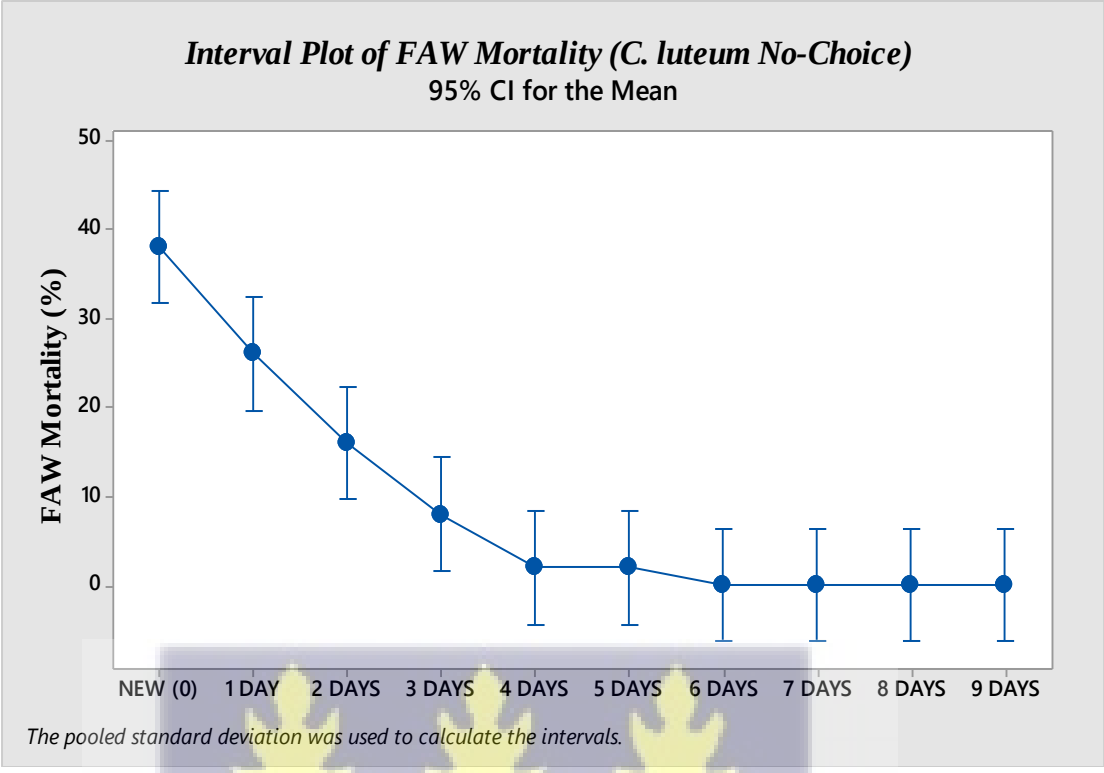
A.



B.

Figure 4.2.2: Percentage adult parasitoid emergence of *C. luteum* (A) and *C. icipe* (B) on different Fall armyworm stages.

A.



B.

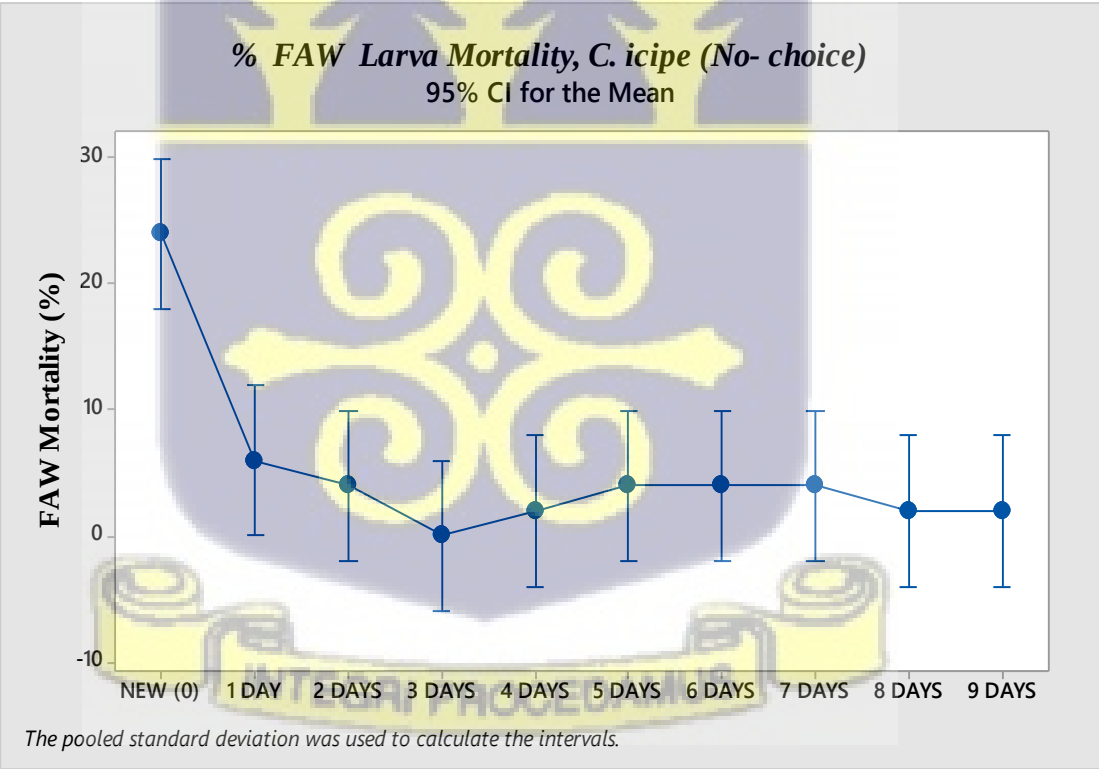


Figure 4.2.3: Mortality effects of *C. luteum* (A) and *C. icipe* (B) parasitism on different Fall armyworm stages.

4.2.2 Choice test

Coccygidium luteum

In the choice test, *C. luteum* recorded an average % parasitism of 20, 40, 40, 60, 80, 80, 40, 0, 0, and 0 % out of 50 newly emerged day 0, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days and 9 days old Fall armyworm larvae, respectively with significant difference between groups (P = 0.016) (Fig. 4.2.4A). Percentage (%) adult parasitoid emergence out of larval parasitoids for newly emerged (day 0) to 9 days old larva was 20, 40, 40, 20, 80, 80, 40, 0, 0, and 0 %, respectively with significant difference between groups (P = 0.016) (Fig. 4.2.5A). Percentage (%) mortality in newly emerged Fall armyworm larvae to 9 days old was 80, 60, 60, 40, 20, 20, 20, 0, 0, and 0%, respectively (P = 0.027) (Table 4.2.2; Fig. 4.2.6A).

Table 4.2.2: Percentage values of *C. luteum* parasitism variables with Different FAW larval stages. Values with the same letter in the column do not differ by Fisher Pairwise Comparisons **(**LSD**)** test.

Fall armyworm age (days)	% parasitism	% Adult parasitoid emergence	% Fall armyworm larva mortality
Newly emerged (0)	20 bc	20 b	80 a
1	40 abc	40 ab	60 ab
2	40 abc	40 ab	60 ab
3	60 ab	20 b	40 abc
4	80 a	80 a	20 bc
5	80 a	80 a	20 bc
6	40 abc	40 ab	20 bc
7	0 c	0 b	0 c
8	0 c	0 b	0 c
9	0 c	0 b	0 c
(ANOVA)	0.016	0.016	0.027

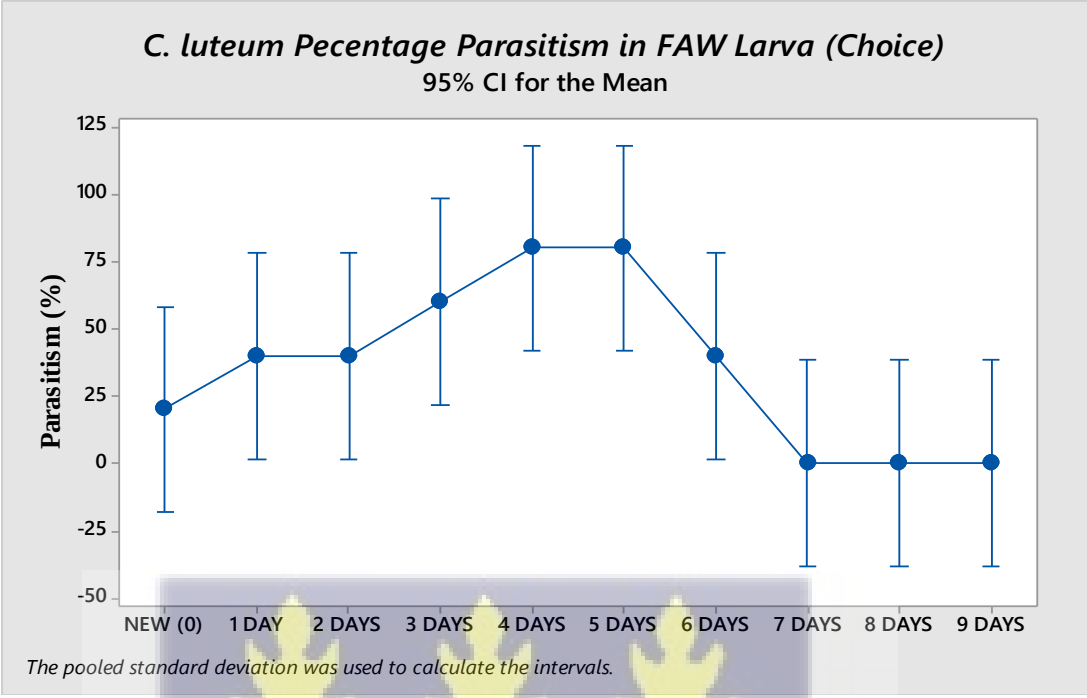
Cotesia icipe

C. icipe on the other hand recorded an average % parasitism of 20, 20, 20, 60, 20, 0, 0, 0, 0, and 0 % out of 50 newly emerged (day 0), 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days and 9 days old Fall armyworm larvae respectively with no significant difference between groups (P = 0.138) (Fig. 4.2.4B). Percentage (%) adult parasitoid emergence out of larval parasitoids for newly emerged day 0 to 9 days old larva was 20, 20, 20, 20, 40, 0, 0, 0, 0, and 0 %, respectively with no significant difference between groups (P = 0.543) (Fig. 4.2.5B). Percentage (%) mortality in newly emerged Fall armyworm larvae to 9 days old was 80, 80, 80, 40, 40, 0, 20, 0, 0, and 0%, respectively with significant difference between Fall armyworm larval stages (days) (P < 0.001) (Table 4.2.3; Fig. 4.2.6B) .

Table 4.2.3: Percentage values of *C. icipe* parasitism variables with Different FAW larval stages. Values with the same letter in the column do not differ by Fisher Pairwise Comparisons (LSD) test.

Fall armyworm age (days)	% parasitism	% Adult parasitoid emergence	% Fall armyworm larva mortality
Newly emerged (0)	20 ab	20 a	80 a
1	20 ab	20 a	80 a
2	20 ab	20 a	80 a
3	60 a	20 a	40 ab
4	20 ab	40 a	40 ab
5	0 b	0 a	0 b
6	0 b	0 a	20 b
7	0 b	0 a	0 b
8	0 b	0 a	0 b
9	0 b	0 a	0 b
(ANOVA)	0.138	0.543	< 0.001

A.



B.

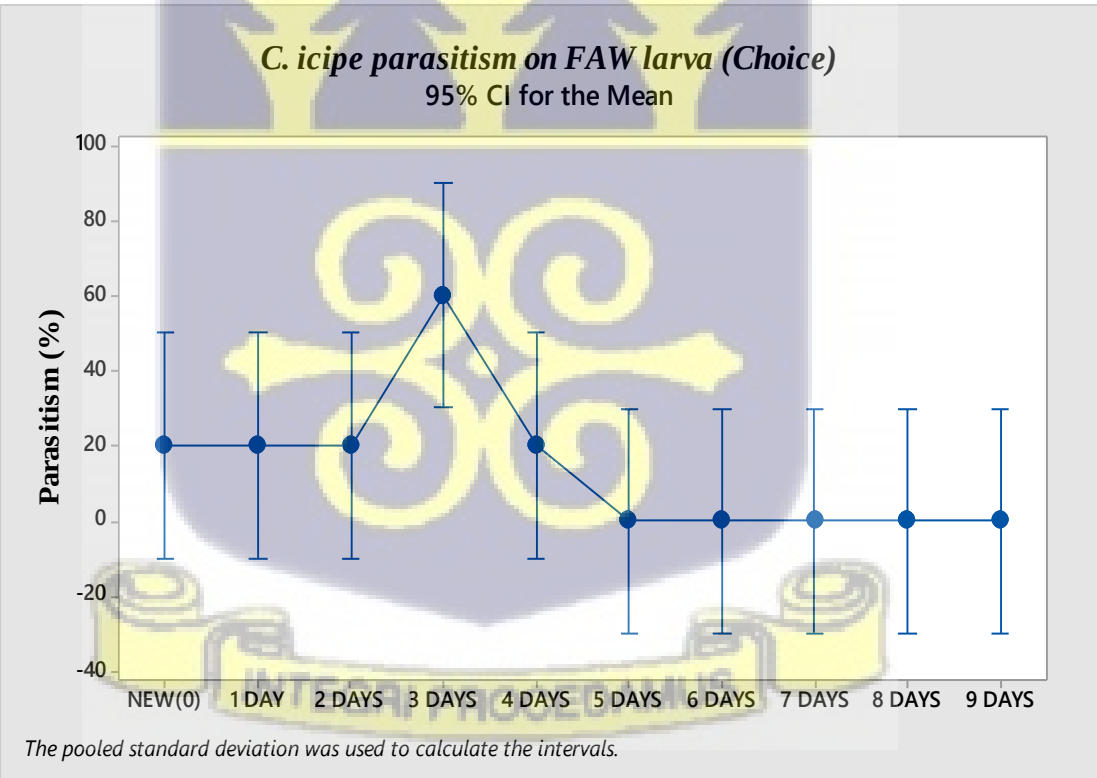
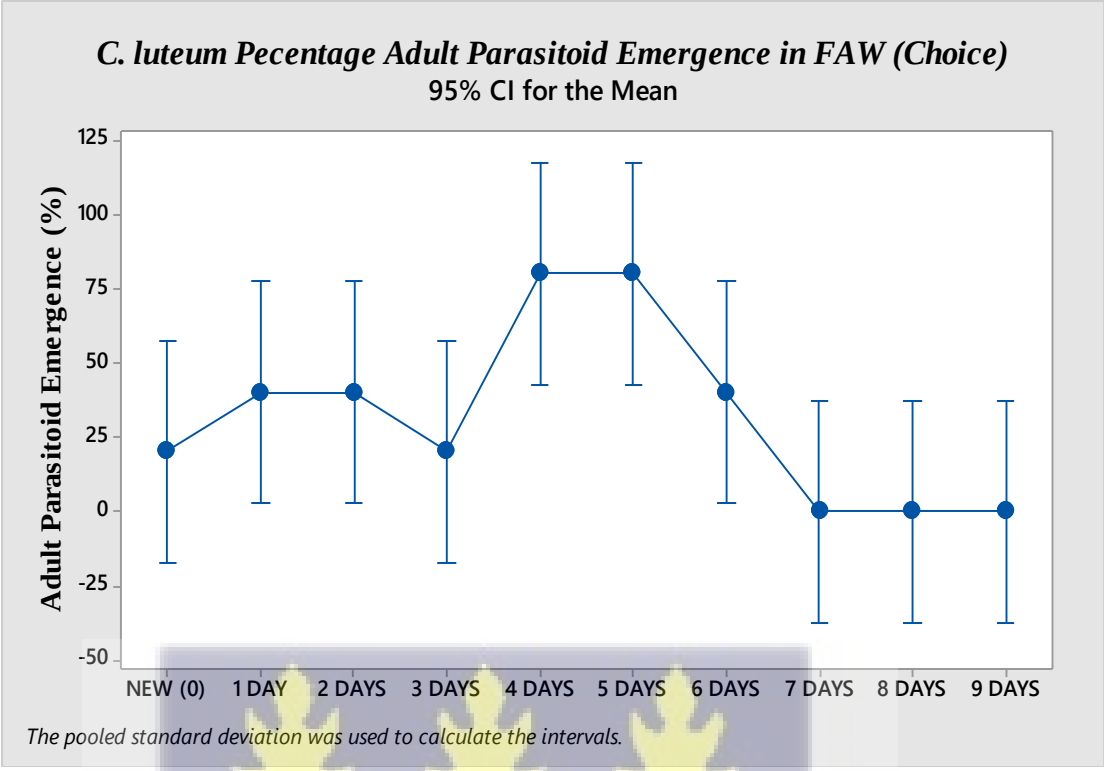


Figure 4.2.4: Parasitism levels of *C. luteum* (A) and *C. icipe* (B) on different Fall armyworm stages in a choice test.

A.



B.

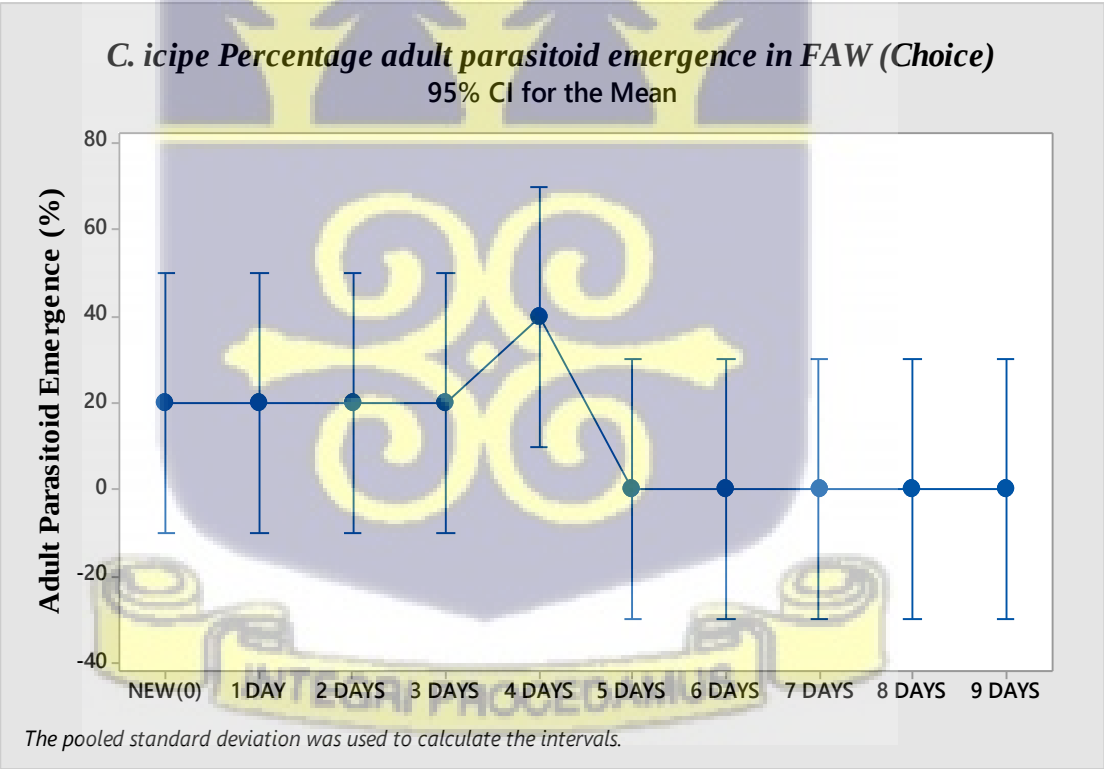
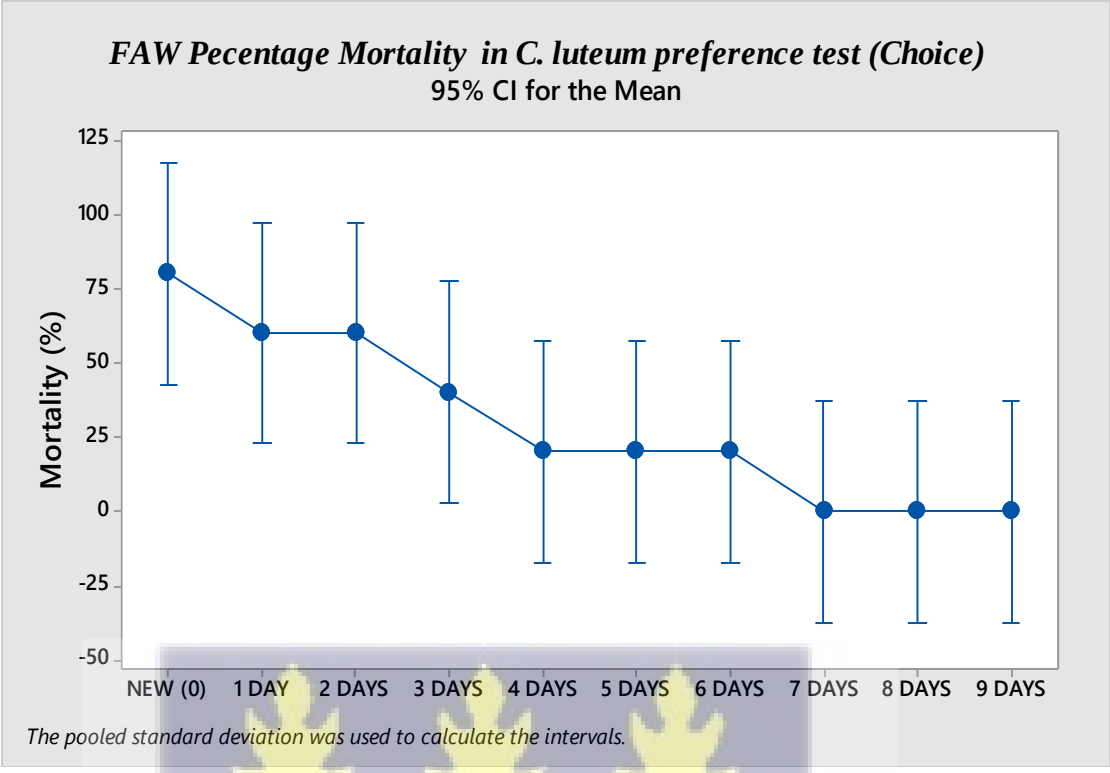


Figure 4.2.5: Percentage adult parasitoid emergence of *C. luteum* (A) and *C. icipe* (B) on different Fall armyworm stages in Choice test.

A.



B.

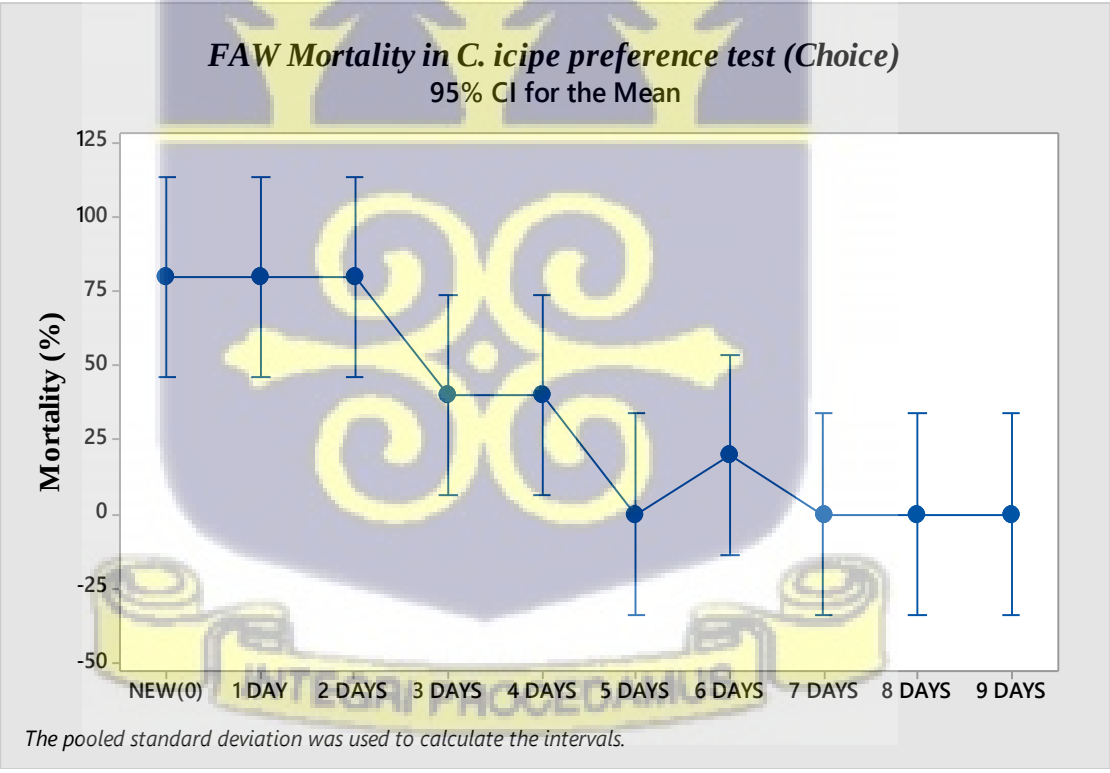


Figure 4.2.6: Mortality effects of *C. luteum* (A) and *C. icipe* (B) parasitism on different Fall armyworm stages in a choice test.

4.2.3 Parasitoid parasitism behaviour and response in Fall armyworm.

C. luteum **(No-choice)**

Behaviour expressions and response by the parasitoids and Fall armyworm was observed in 50 Fall armyworm larvae and a female parasitoid. In *C. luteum* against Fall armyworm, all 50 newly emerged larvae day 0, one day (1), two days (2) and three (3) days old larvae were attacked (probed) by *C. luteum* and did not express a defensive response (**AND**: Attack No Defense), representing 100%. In the case of four (4) to six (6) days old Fall armyworm larva, attack and no defense behaviour was 88, 30, and 2 %, respectively, while seven (7) days to nine (9) days was 0 % ($P < 0.001$).

For parasitoid attempt (attempted probing) and defensive (aggression) behaviour in Fall armyworm larva observed (**AD**: Attack but Defensive), newly emerged day 0 Fall armyworm larva, one (1) to three (3) days old larva recorded zero percent (0%), while four (4), five, six, seven, eight, and nine days old larva recorded 12, 70, 98, 88, 62, and 18 %, respectively ($P < 0.001$).

In the no parasitism attempt **(NA**) behaviour observations, newly emerged (day 0), 1 day to 5 days old recorded zero percent (0%), while six, seven, eight and nine days old Fall armyworm larva recorded 2, 12, 38, and 82 %, respectively ($P < 0.001$) (Fig. 4.2.7 A).

***C. icipe* (No-choice)**

In *C. icipe* no-choice parasitism behaviour and response in Fall armyworm larvae, newly emerged day 0 and one (1) day old Fall armyworm larva recorded zero percent (0%), while two days to nine days old Fall armyworm larva recorded 6, 18, 22, 42, 60, 84, 96, and 96 %, respectively in no parasitism attempt **(NA)** with significant difference between stages (ages) of Fall armyworm ($P < 0.001$).

There was zero (0) % attack but defensive **(AD)** behaviour in newly emerged (day 0) and one (1) day old Fall armyworm larva, while two (2), three (3), four (4), five (5), six (6), seven (7), eight (8) and nine (9) days old recorded 10, 24, 74, 58,40, 16, 4, and 4 % attack but defensive behaviour **(AD)**, respectively with a significant difference between Fall armyworm ages ($P < 0.001$).

Cotesia icipe recorded 100 % attack and no defense **(AND)** behaviour in newly emerged (day 0) and one (1) day old Fall armyworm larva. While two (2), three (3), and four (4) days larva recorded 84, 58, and 4 %, respectively. Five (5), six (6), seven (7), eight (8) and nine (9) days old larva on the other hand recorded zero (0) % attack and no defense **(AND)** behaviour ($P < 0.001$) (Fig. 4.2.7B).

A.

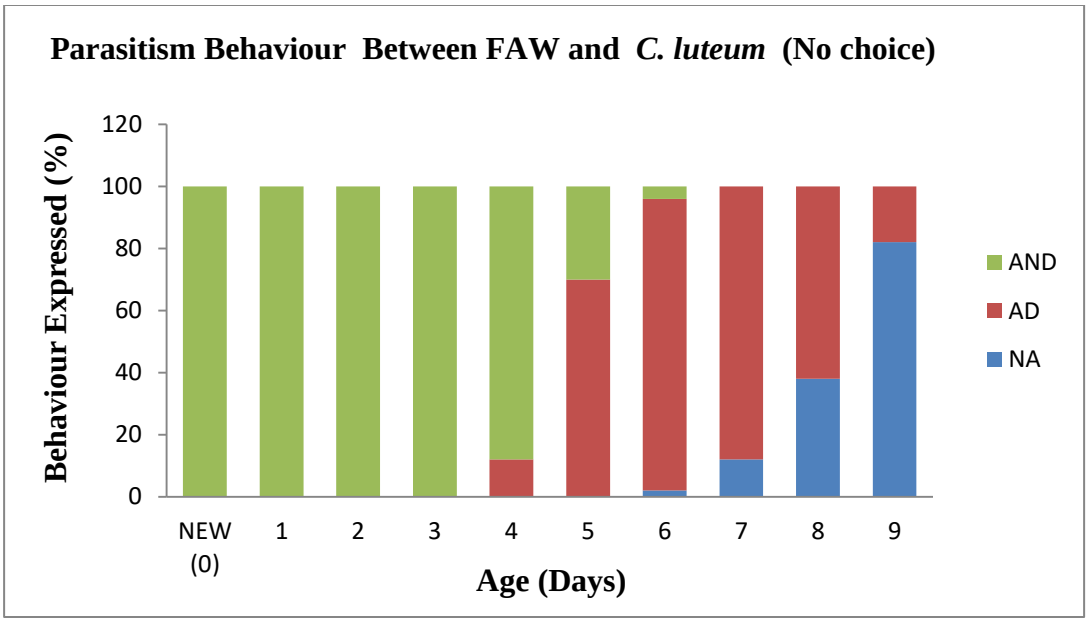


Figure 4.2.7 A: Parasitism behaviour of *C. luteum* and FAW response in no-choice test.

B.

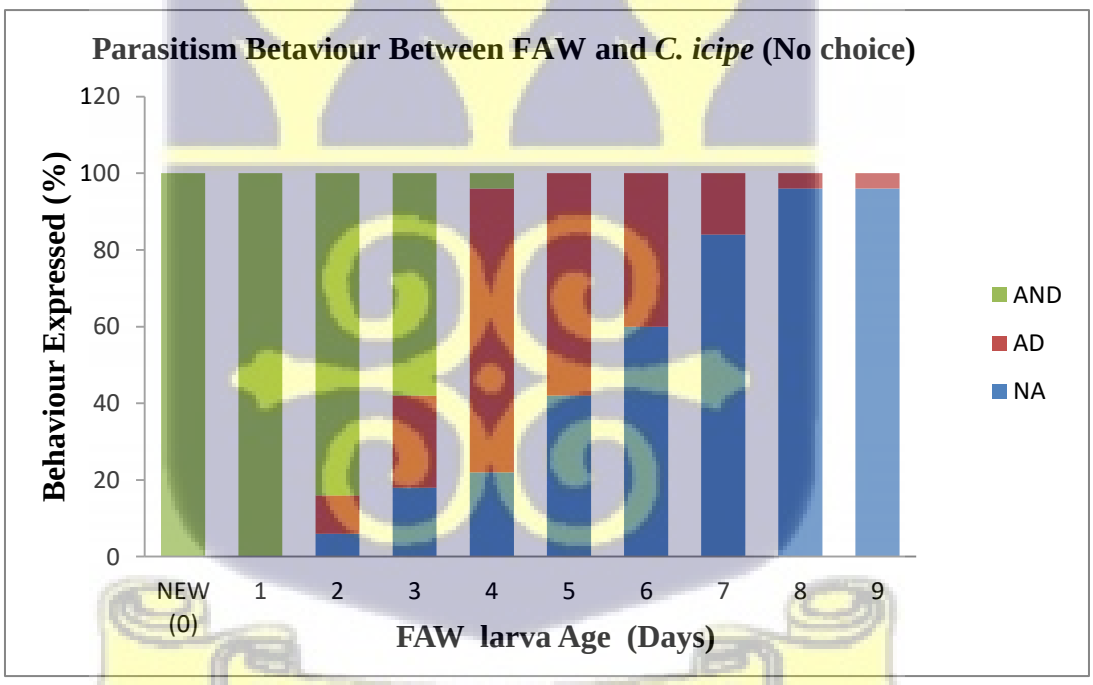


Figure 4.2.7 B: Parasitism behaviour of *C. icipe* and FAW response in no-choice test.

Choice test

C. luteum

In *C. luteum* parasitism on Fall armyworm choice test, all 50 newly emerged larvae (day 0), one day (1), two days (2), three (3), and four (4) days old larvae were attacked (probed) by *C. luteum* and also did not express a defensive response (**AND**: Attack No Defense), representing 100% behavioural action and response. Also, five (5) to six (6) days old Fall armyworm larva, attack and no defense behaviour was **(AND)** 0 % with significant difference between Fall armyworm stages ($P < 0.001$).

For *C. luteum* attempt (probing) and defensive (aggression) behaviour in Fall armyworm larva observed (**AD**: Attack but Defensive), all the Fall armyworm larva stages recorded zero percent (0%) , except five, six and seven days old larva which recorded 80, 60, and 40 %, respectively ($P < 0.001$).

In the no parasitism attempt **(NA**) behaviour observations, newly emerged day 0, one day to four days old Fall armyworm larva recorded zero percent (0%) behaviour expression, while five, six, seven, eight and nine days old Fall armyworm larva recorded 20,40,60 and 100 % for both eight and nine days old Fall armyworm larva respectively ($P < 0.001$) (Fig. 4.2.8A).

C. icipe

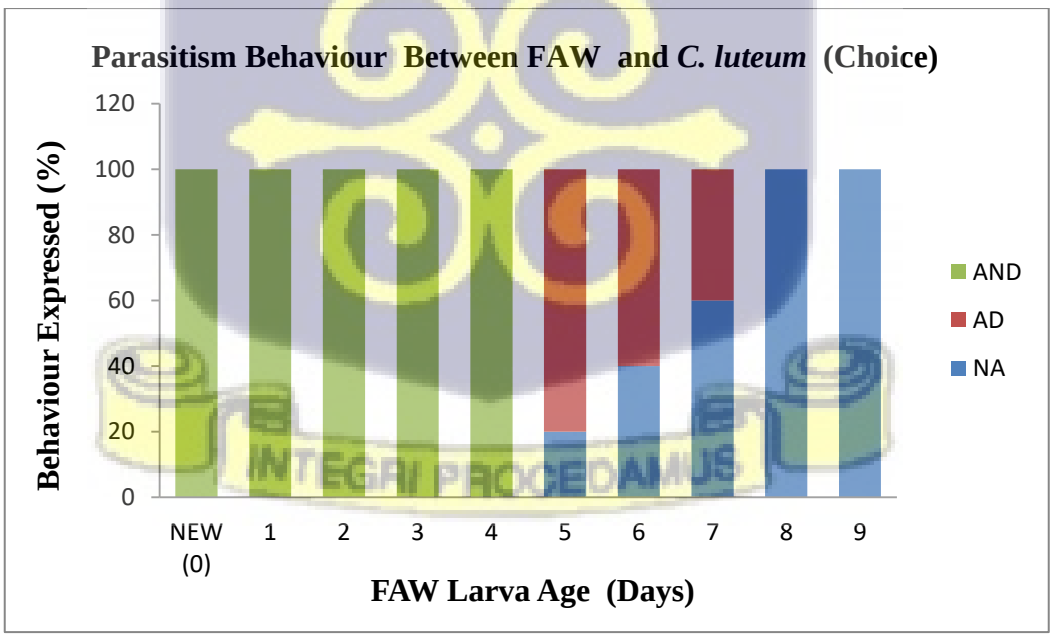
In the case of *Cotesia icipe* parasitism on Fall armyworm choice test, all fifty (50) newly emerged larvae (day 0), one day (1), two days (2), and three (3) days old larvae were attacked (probed) by *C. icipe* with no defensive response from Fall armyworm (**AND**:

Attack No Defense), representing 100% behavioural action and response. Four days to 9 days old Fall armyworm larva, attack and no defense behaviour **(AND)** was 0 % with significant difference between Fall armyworm stages ($P < 0.001$).

For *C. icipe* probing attempt and defensive (aggression) behavior in Fall armyworm larva (**AD**: Attack but Defensive), all the Fall armyworm larva stages expressed no attack but defensive behaviour (0%) , except four, five and six days old larva recorded 40, 40, and 20 % respectively ($P = 0.097$).

In the no parasitism attempt **(NA**) behaviour observations, newly emerged (day 0), one day to three days old Fall armyworm larva expressed zero percent (0%) no parasitism attempt **(NA)** behaviour, while four, five, six, seven, eight and nine days old Fall armyworm larva recorded 60, 60, 80 and 100 % for seven, eight and nine days old Fall armyworm larva respectively ($P < 0.001$) (Fig.4.2.8B).

A.



B.

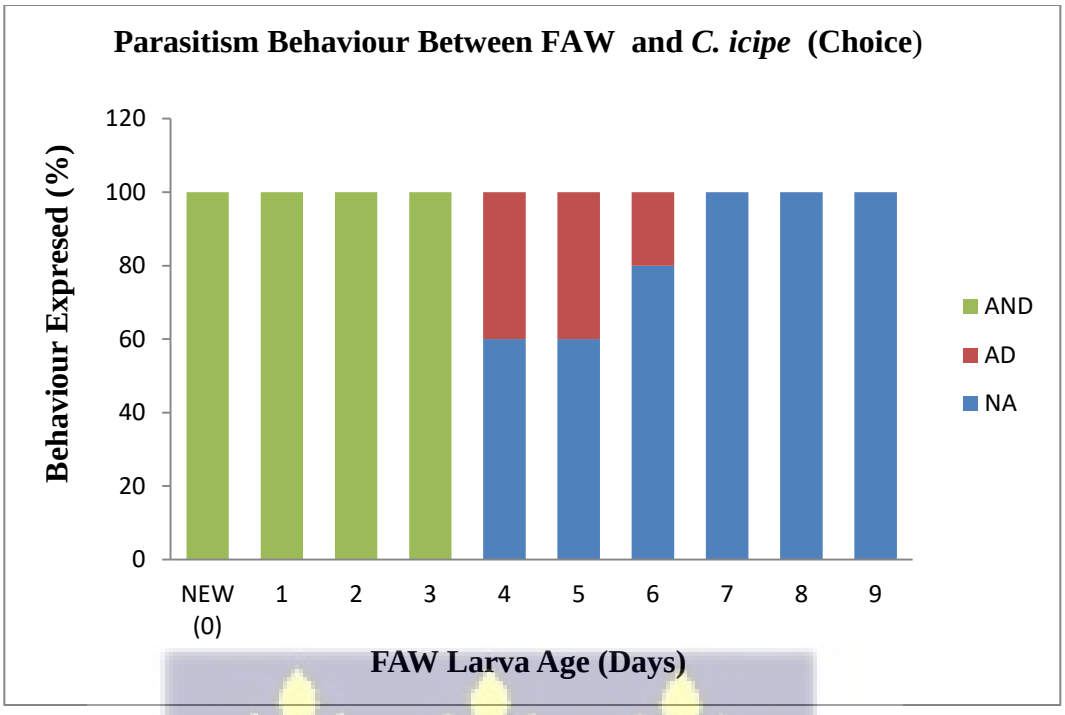


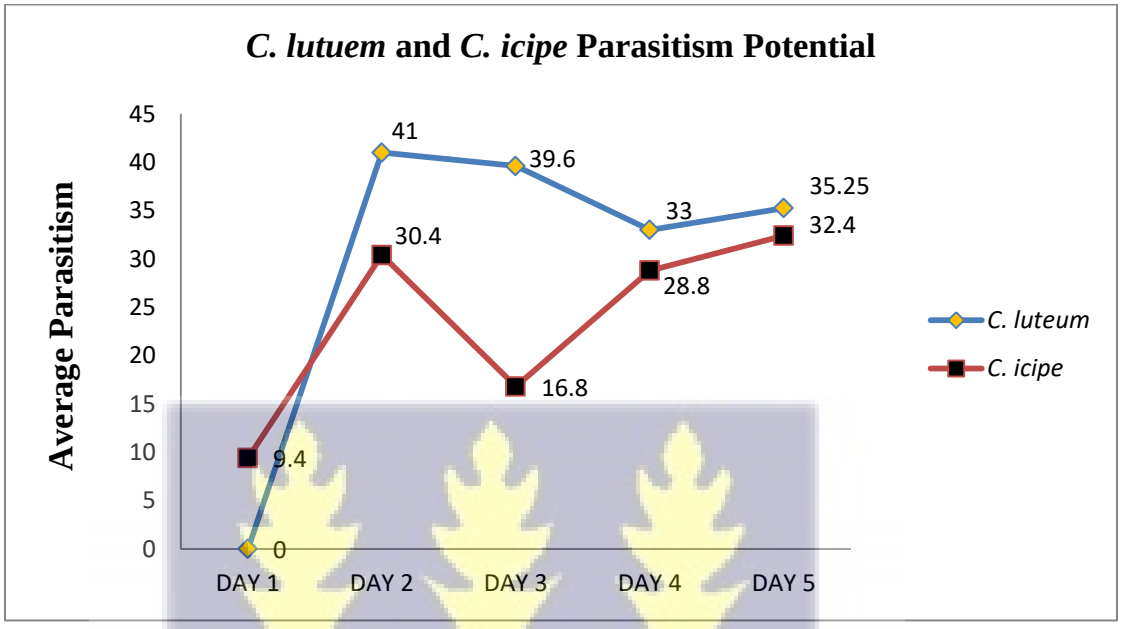
Figure 4.2.8: Parasitism behaviour of *C. luteum* (A) and *C. icipe* (B) against FAW response in choice test.

4.3 Parasitism potential

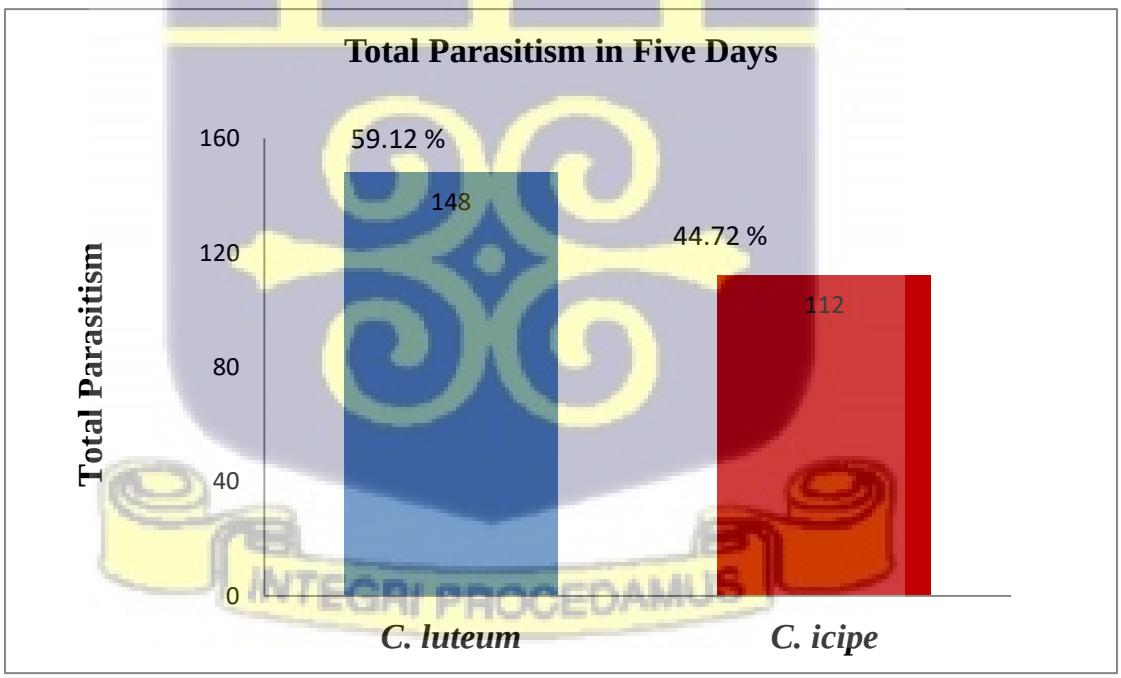
Average daily parasitism in *C. luteum* was 0, 41, 40, 33, and 35 for day one, day two, day three, day four, and day five, respectively, representing 0, 82, 79, 66, and 71 %, respectively. There was significant difference in the average daily parasitism ($P < 0.001$) (Fig. 4.3A).

Cotesia icipe on the other hand recorded 10, 31, 17, 29, and 33 daily parasitim for day one, day two, day three, day four, and day five, respectively, representing 19, 61, 34, 46, and 65 %, respectively. The means were significantly not the same ($P < 0.001$) (Fig. 4.3A).

Total parasitism of *C. luteum* and *C. icipe* was 148 and 112, which represent 59.12 % and 44.72 %, respectively, with no significant difference in total parasitism (P = 0.507) (Fig. 4.3B).



A.



B.

Figure 4.3: Average daily parasitism potential (A), and total parasitism potential (B).

4.3.1 Parasitism effect on Fall armyworm mortality

Coccygidium luteum average percentage mortality for day one, two, three, four, and day five was 4.2, 3.3, 2, 1.6, and 3 %, respectively with a partial no significant difference in daily percentage mortality (P = 0.046). *C. icipe* recorded 23.6, 4.4, 1.8, 5.6, and 5.6 % for day one, two, three, four, and day five, respectively with a significant difference between daily mortality means (P < 0.001).

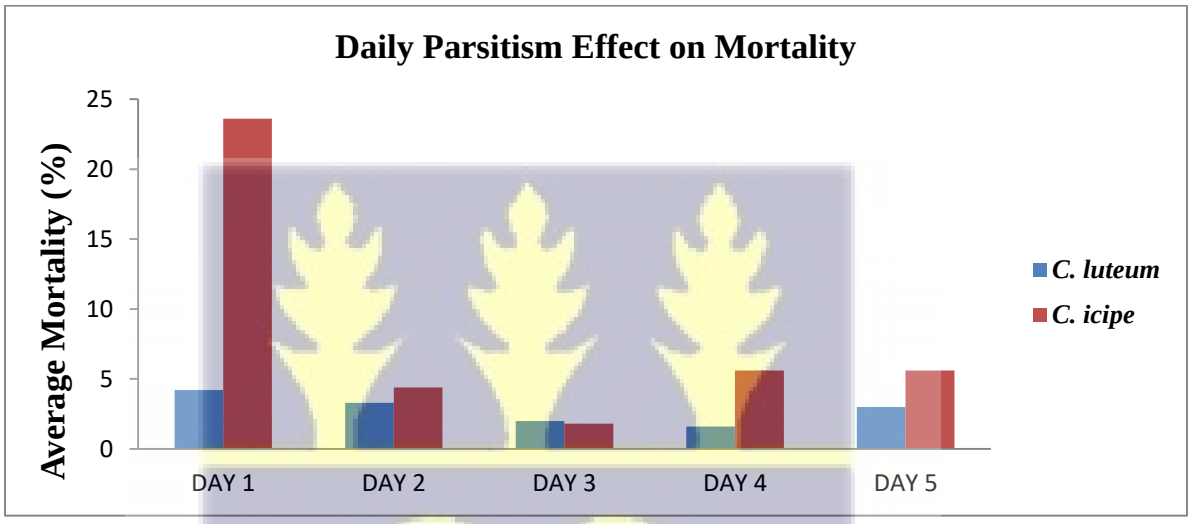


Figure 4.3.1: Average daily FAW mortality during parasitism.

Figure 4.3.2: Dead Fall armyworm larvae after parasitism (probing).

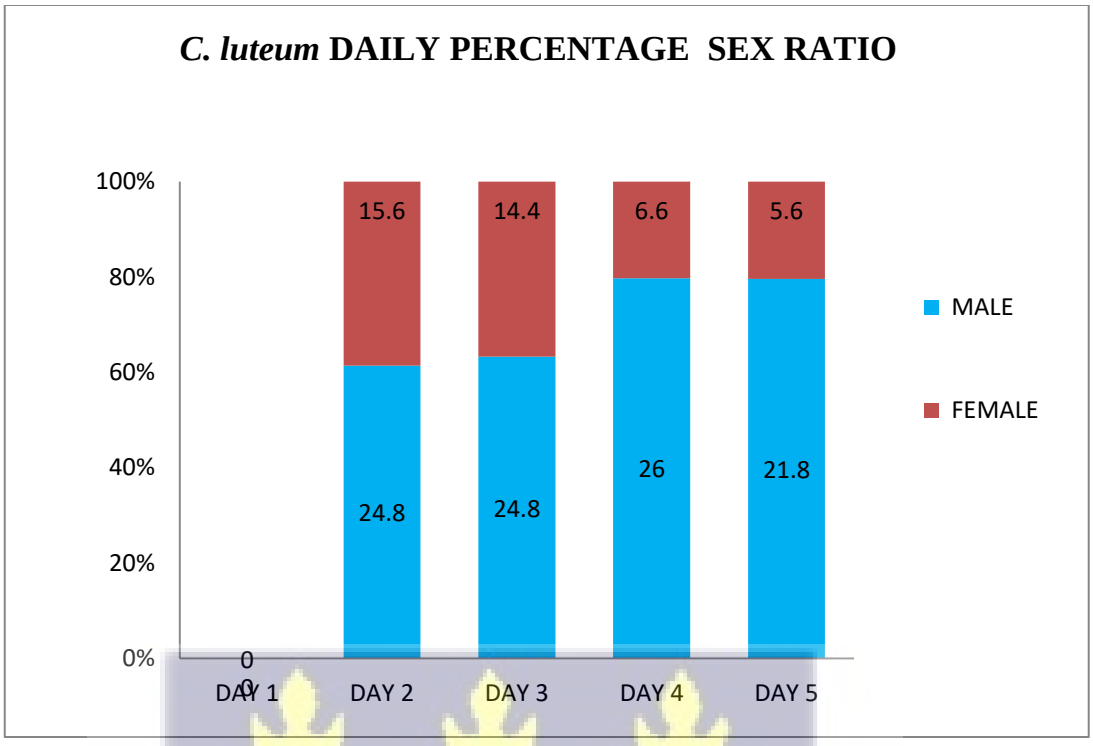
4.3.2 Sex ratio of *C. luteum* and *C. icipe*

Coccygidium luteum recorded daily average of 0, 16, 15, 7, and 6 females in day one, two, three, four and five, representing 0, 39, 37, 20 and 20 %, respectively. The males were 0, 25, 25, 26, and 22 for day one, two, three, four and five, representing 0, 61, 63, 80 and 80%, respectively (Table 4.3; Fig. 4.3.2A). *Cotesia icipe* recorded daily average of 9, 12, 8, 10, and 12 males in day one, two, three, four, and five, representing 94, 38, 48, 43 and 36 %, respectively whereas females recorded 1, 19, 9, 13, and 21 representing 6, 62, 52, 57 and 64 %, respectively (Table 4.3; Fig. 4.3.2B). The average total male and female parasitoids recorded in Five (5) days was 98 and 42, representing 70 and 30 %, respectively for *C. luteum*, while *C. icipe* recorded 50 and 62, representing 45 and 55 % male and females, respectively (Table 4.3; Fig. 4.3.3). Sex ratio was 1 male to 1.3 females (1 M♂: 1 F♀) and 2.3 males to 1 female (2 M♂: 1 F♀) for *C. icipe* and *C. luteum*, respectively.

Table 4.3: Average and total values for parasitoids potentials with sex ratio

	C. luteum **(mean ± SE mean)**		***C. icipe*** **(mean ± SE mean)**	
DAY	MALE(♂)	FEMALE(♀)	MALE(♂)	FEMALE(♀)
1	0	0	8.80 ± 2.52	0.60 ± 0.40
2	24.80 ± 2.37	15.60 ± 1.57	11.60 ± 1.25	18.80 ± 1.46
3	24.80 ± 3.54	14.40 ± 2.18	8.000 ± 0.316	8.80 ± 1.24
4	26.00 ± 3.51	6.60 ± 1.17	9.800 ± 0.735	13.00 ± 0.84
5	21.80 ± 5.52	5.60 ± 1.78	11.60 ± 1.36	20.80 ± 2.35
TOTAL	97.40 ± 6.67	42.20 ± 3.50	49.80 ± 2.08	62.00 ± 4.09
SEX RATIO	**(2.3 ♂: 1 ♀)**		**(1 ♂: 1.3 ♀)**	

A.



B.

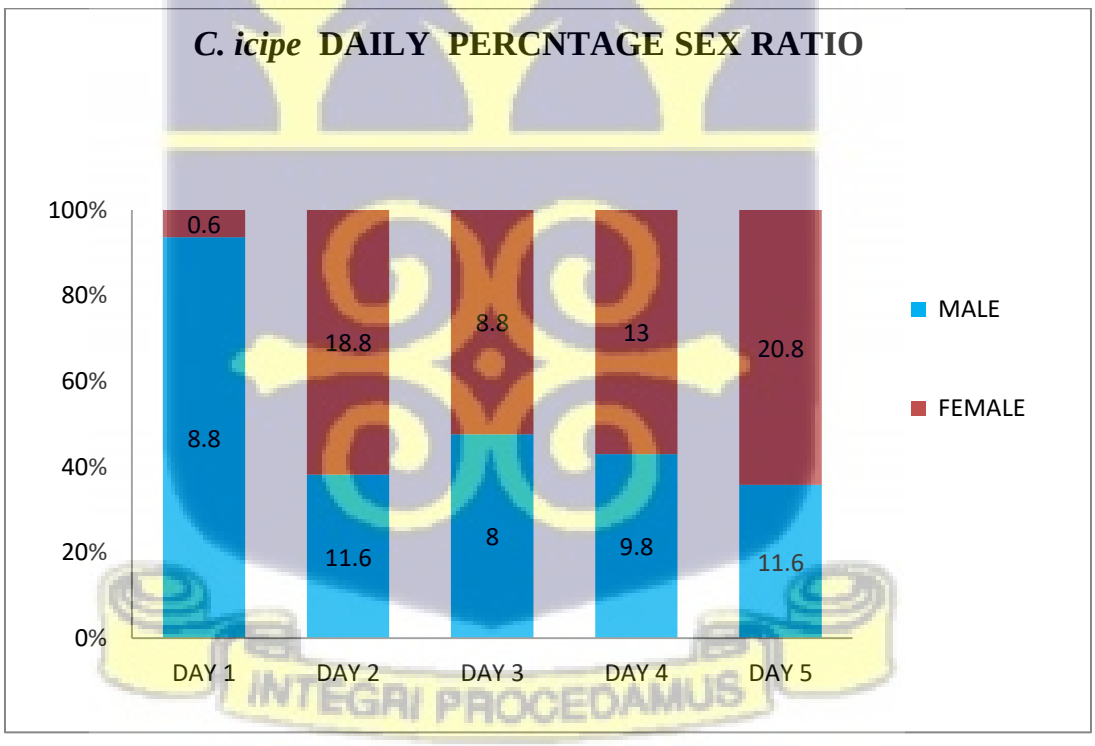


Figure 4.3.2: Average daily percentage of male and female *C. luteum* (A) and *C. icipe* (B).

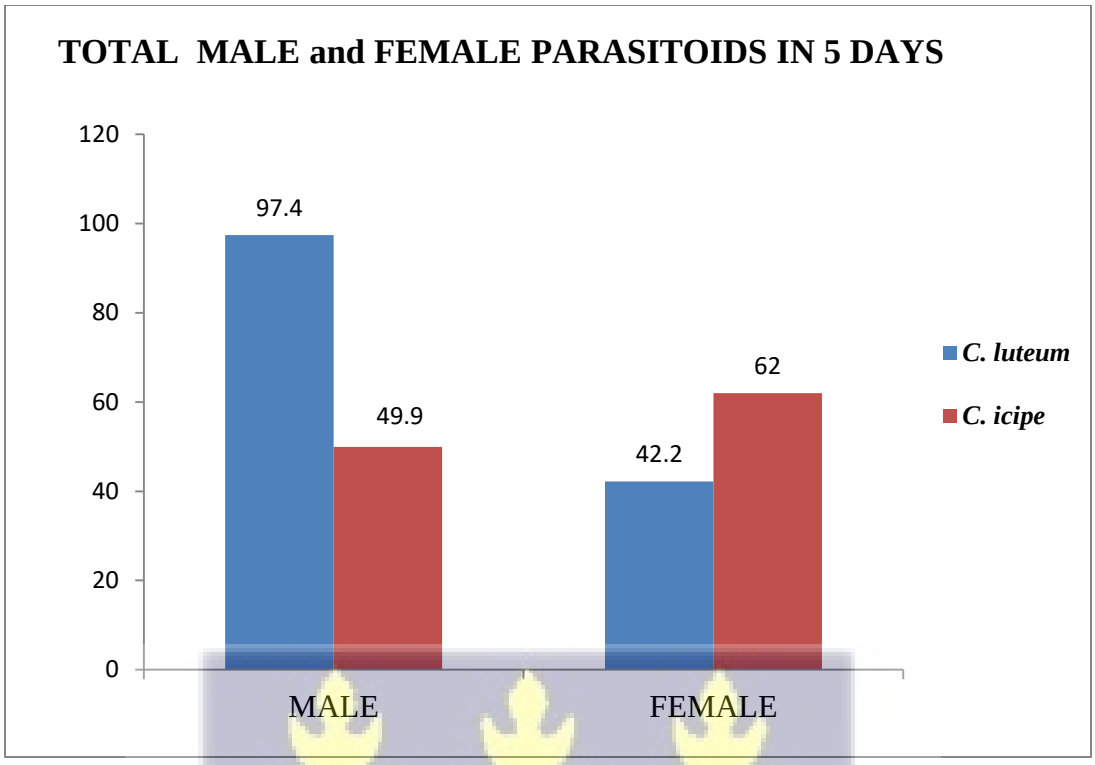


Figure 4.3.3: Total male and female parasitism potentials of *C. luteum* and *C. icipe*.

4.4 Parasitism interaction between *C. luteum* and *C. luteum* in Fall armyworm

4.4.1 Sequential interaction

In introduction of Fall armyworm larvae first to *C. luteum* before later introduction of same larvae to *C. icipe*, a total of 36 and 44 which represents 24 % and 29.4 % for *C. luteum* and *C. icipe* respectively emerged from Fall armyworm larva. Mortality was 69 representing 46 %. The first introduction of larvae to *C. icipe* before *C. luteum* also recorded a higher number of *C. icipe* than *C. luteum* with a total of 35 and 42, representing 23.4 % and 28 % for *C. luteum* and *C. icipe* respectively. Mortality was 68 representing 45.4 %. There was no significant difference between *C. icipe* and *C. luteum*

emergence in both *C. icipe* first (P = 0.357) and *C. luteum* first (P = 0.298) sequential introduction (Fig. 4.4.1).

4.4.2 Concurrent interaction

In the introduction of Fall armyworm to both parasitoids at the same time in a cage, a total of 46 and 19 parasitism was recorded, representing 30.7 % and 12.7 % for *C. luteum* and *C. icipe*, respectively with significant difference in total parasitoid emergence (P < 0.001) (Fig. 4.4.1). Mortality was 66, representing 44 % out of 150 Fall armyworm larvae.

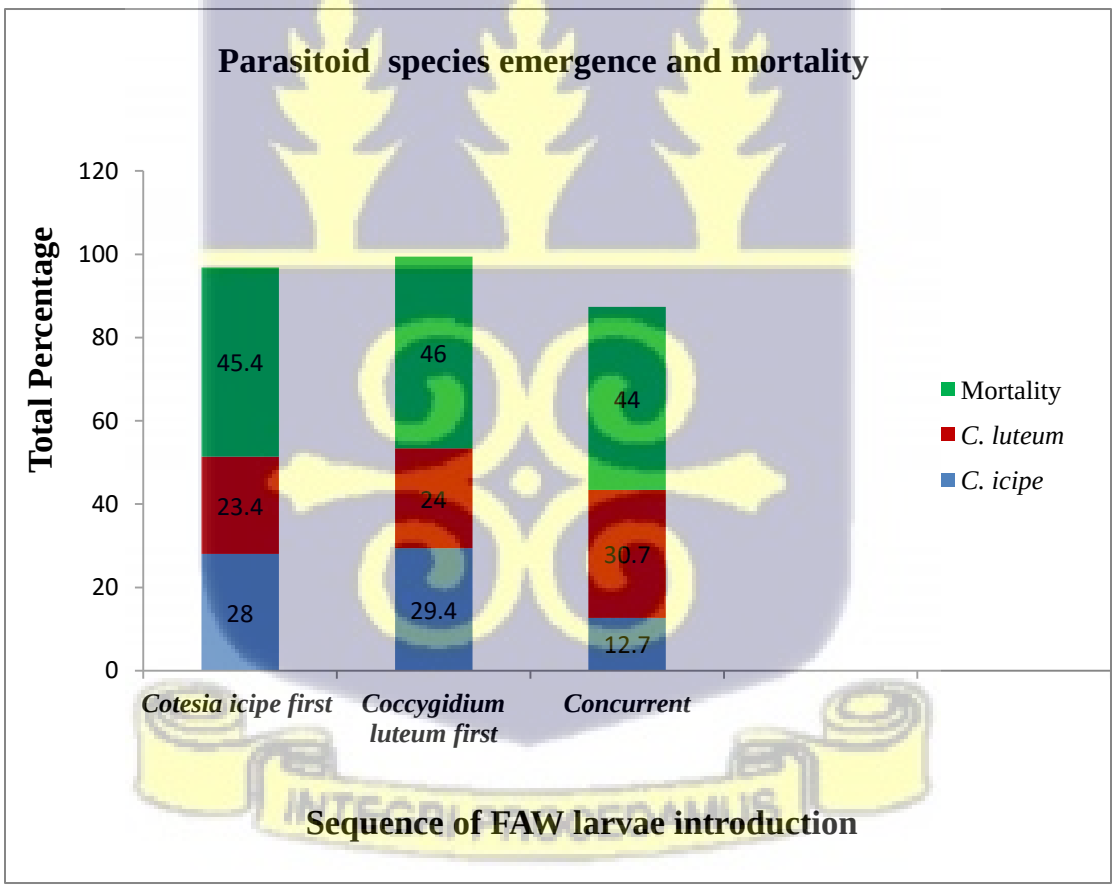


Figure 4.4.1: *C. luteum* and *C. icipe* emergence and mortality in Sequential introduction.

4.4.3 Comparing all parasitoid introduction grouping (sequential, concurrent, control)

There was a significant difference in *C. icipe* emergence in all test grouping (introducing *C. icipe* 1st, *C. luteum* 1st, concurrently and only *C. icipe*) ($F = 120.78$; $P < 0.001$). Percentage *C. icipe* parasitism was 28, 29.4, 12.7, and 91.4 % for *C. icipe* 1st, *C. luteum* 1st, concurrent introduction and only *C. icipe* introduction, respectively. There was also a significant difference in *C. luteum* emergence in all test grouping (introducing *C. luteum* 1st, *C. icipe* 1st and concurrently) ($F = 61.90$; $P < 0.001$). Percentage *C. luteum* parasitism was 24.4, 24, 31, and 81 % for *C. icipe* 1st, *C. luteum* 1st, concurrent introduction and only *C. luteum* introduction, respectively (Table 4.4.1; Fig. 4.4.2).

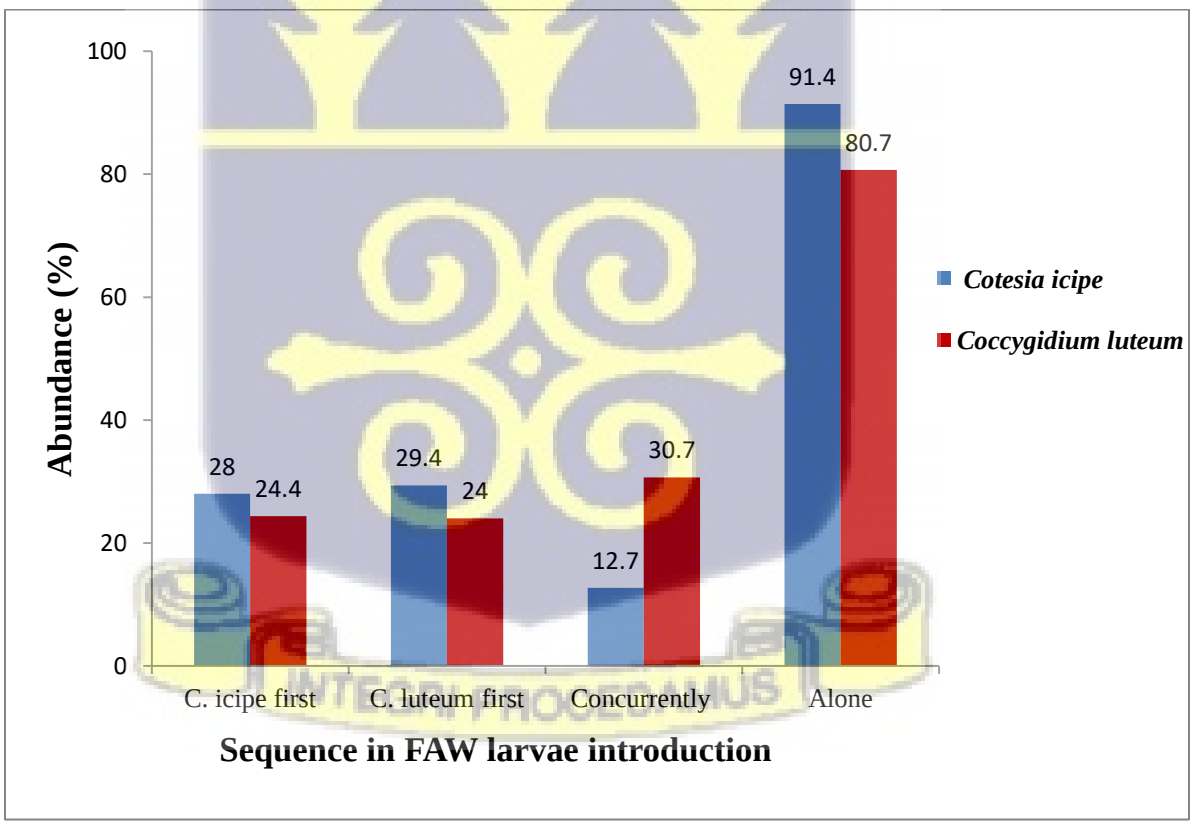


Figure 4.4.2: *C. luteum* and *C. icipe* abundance in all FAW larvae introduction sequence.

Table 4.4.1: Percentage parasitism in test groupings and significance mean separation

Introduction sequence	**Total *Cotesia icipe***	**Total *Coccygidium luteum***
C. icipe first	28.0 % **b**	24.4 % **b**
C. luteum first	29.4 **%** **b**	24.0 % **b**
Concurrently	12.7 % **c**	30.7 % **b**
Alone	91.4 % **a**	80.7 % **a**
Significance level	**(F= 120.78; P < 0.001)**	**(F= 61.90; P < 0.001)**

There was 53.4, 51.4, 43.4, 80.7, and 91.4 % total parasitism in introducing *C. luteum* 1st, *C. icipe* 1st, concurrent and negative control (*C. luteum* alone and *C. icipe* alone) respectively, with significant difference between means (F = 32.83; P- value < 0.001) (Table 4.4.2; Fig. 4.4.3).

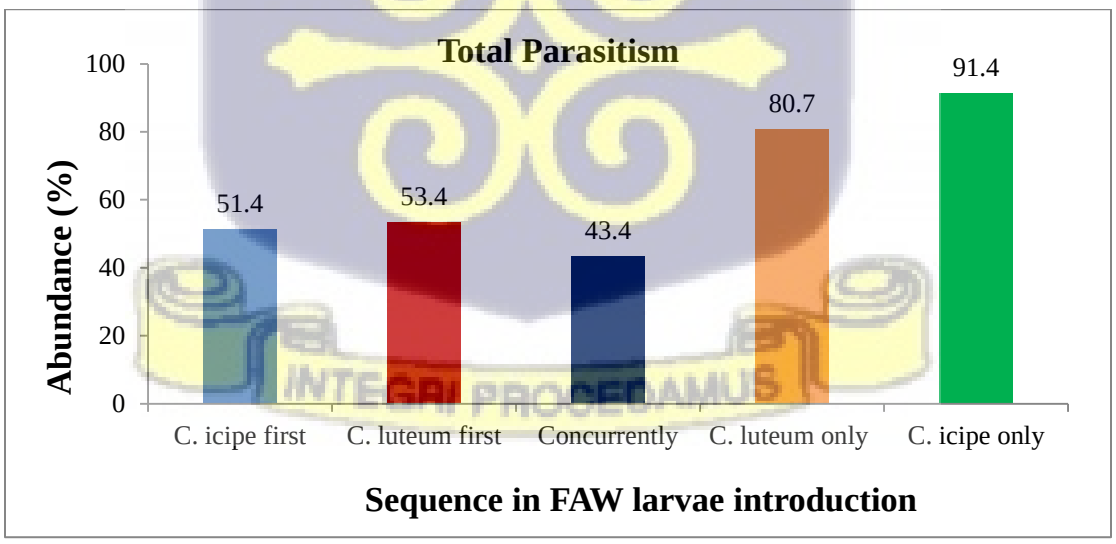


Figure 4.4.3 Total percentage parasitism in all FAW larvae introduction sequence.

Total Fall armyworm mortality on the other hand was 46, 45.4, 44.3, 17.4, 4.7, and 2.0 % for *C. luteum* 1st, *C. icipe* 1st, concurrent, negative control (*C. luteum* alone and *C. icipe* alone) and positive control (no FAW larvae exposure to parasitoids) respectively with significant difference between groups ($F = 41.61$; $P < 0.001$), (Table 4.4.2; Fig. 4.4.4).

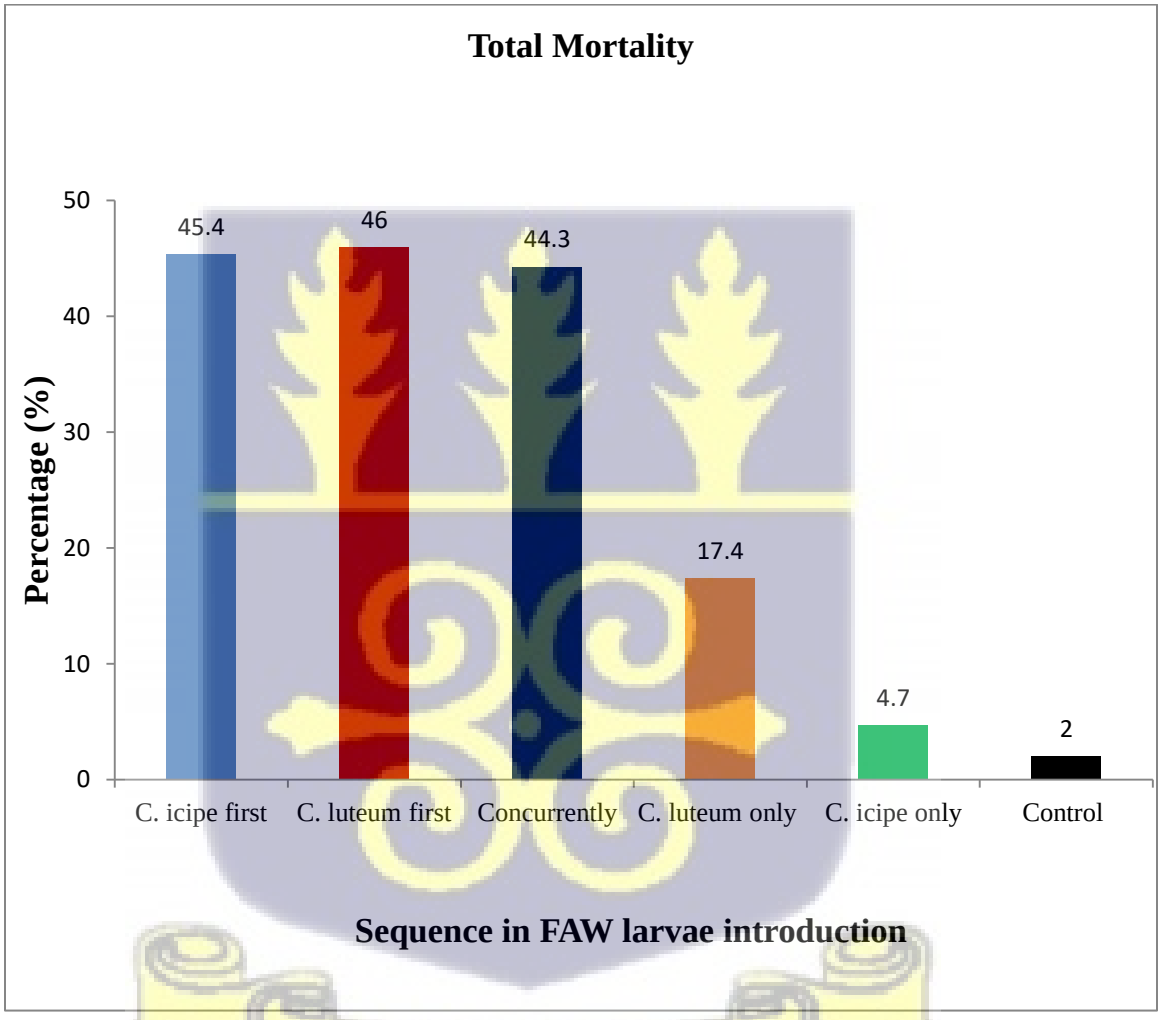


Figure 4.4.4: Total percentage Fall armyworm mortality in all introduction sequence.

Table 4.4.2: Total percentage parasitism and mortality in interaction test groupings.

Parasitoid Introduction	**Total Parasitism**	**Total Mortality**
C. icipe first	51.4 % **b**	45.4 % **a**
C. luteum first	53.4 % **b**	46.0 % **a**
Concurrently	43.4 % **b**	44.3 % **a**
C. luteum only	80.7 % **a**	17.4 % **b**
C. icipe only	91.4 % **a**	4.7 % **b c**
Control	-------	2.0 % **c**
Significance level	**(F= 32.83 ; P < 0.001)**	**(F= 41.61; P < 0.001)**

CHAPTER FIVE

5.0 DISCUSION

5.1 Parasitoid life span

The adult male and female *Cotesia icipe* and *Coccygidium luteum* mate shortly after emergence. During mating the males are always positioned on top of the female while flapping their wings. The female *C. icipe* oviposit on the same day of emergence as observed in the study within 12 hours after emergence, while *C. luteum* on the other hand oviposit after 24 hours of emergence. The period for *C. luteum* development from egg to larval emergence in the Fall armyworm larva was 9 days, which was by far greater than that of *C. icipe* at 7 days. The average duration of parasitoid larva to pupa was 8 hours in *C. luteum* as compared to 4 hours in *C. icipe* which was two times lesser. The duration of pupa to adult was the second shortest developmental stage after the larval to pupal stage in *C. icipe*, at an average of 3 days as compared to 10 days in *C. luteum*, respectively. This phenomenon could be justified with the observation of pseudo legs and partially developed eyes in the *C. icipe* larva before cocoon formation during pupation as compared to *C. luteum* without these morphological characteristics. Generally as observed in the study, male *C. luteum* emerged earlier than female. In all the developmental stages of the two parasitoids, the egg to larva stage was the longest in *C. icipe*, contributing up to 69 % of the entire developmental duration, while in *C. luteum*, pupa to adult stage contributed the most to the total developmental time at 52 %. Adult parasitoid life span was greater in *C. luteum* than in *C. icipe*, where both parasitoids recorded 7 days minimum and 15 days maximum adult life span in *C. icipe* and 12 days minimum and 23 days maximum for *C. luteum*, respectively. *C. icipe* spent most of its

development time at the egg to larva stage while, *C. luteum* on other hand spent most of its development time at the pupa to adult Stage. The developmental time from egg to adult emergence in general was longer in *C. luteum* (19 days) which is larger in size, as compared to *C. icipe* (10 days) of 2.20–2.50mm adult length (Fiaboe *et al*., 2017; Fernandez-Triana *et al*., 2014**).** This conforms to findings where a positive correlation was found to exist between parasitoid body size and host body size in solitary species, as development time is known to be longer for parasitoids attacking larger host which is also a known observation for koinobionts (Blackburn, 1991a). This phenomenon follows the principle of minimizing time spent in vulnerable life- history stages (Stearns, 1992). *Cotesia icipe* in this case is expected to have a shorter development time than *C. luteum*, as it is smaller and exposed to greater mortality risks as they develop in smaller larval host. Also parasitoids whose eggs mature upon emergence (pro-ovigenic) as *C. icipe* and as described in Mohamed *et al.* 2021, where they were found to have eggs matured on the first day of emergence, have a shorter life-span than synovigenic ones as *C. luteum* which agrees with findings from life-span test of Braconidae (Jervis *et al*., 2001). The average generation time was 30 days and 17 days for *C. luteum* and *C. icipe*, respectively. Although the generation time is smaller in *C. icipe*, it has a higher voltinism of 15 minimum and 21 generations maximum in a year as compared to 8 to 12 generations, respectively for *C. luteum*. This implies both parasitoid species are multivoltine species. The general generation time of *C. luteum* did not diverge much from that described by Agboyi *et al.* 2019, where average temperature was above 28 °C. The duration before mating, oviposition and adult life span was however not studied in that test. The actual

larval to pupal stage duration was not obtained, as the larvae to pupae duration was recorded as 1 day (24 hours), with data recorded after a day interval.

5.2 Parasitoids Fall armyworm stage preference

Different Fall armyworm larva stages were introduced to *Cotesia icipe* and *Coccygidium luteum* parasitoids, and the percentage parasitoid larval emergence, percentage adult parasitoid emergence and Fall armyworm mortality after parasitism were used as bases to rank Fall armyworm larva stage preference in a choice and no choice test. In the no-choice test, *C. icipe* recorded 76, 94, 92, 58, 20, 6, 0, 0, 0, and 0 percentage parasitism (parasitoid larva emergence) and 82.7, 93.8, 100.0, 97.2, 80.0, 40.0, 0.0, 0.0, 0.0, and 0.0 percentage adult parasitoid emergence out of the parasitoid larvae in newly emerged Fall armyworm larva (day 0) to nine days old Fall armyworm larvae respectively. However mortality levels varied, as newly emerged Fall armyworm larva day 0, to nine days old Fall armyworm larvae recorded 24.0, 6.0, 4.0, 0.0, 2.0, 4.00, 4.0, 4.0, 2.0, and 2.0 percentage mortality, respectively. Cotesia species are known for host feeding which causes host death (Jervis *et al*., 2001). According to the results obtained on percentage parasitism, *Cotesia icipe* in the no-choice test showed a range of Fall armyworm larva stage preference from newly emerged to four days old larva; 1st to 2nd larva instars according to Pitre and Hogg (1983), with only 6 percent parasitism in five days old larvae. The highest preference was recorded in one day old larvae at 94 percentage parasitism; 1st larva instar (Pitre and Hogg 1983), with two days, day zero, three days and four days Fall armyworm larva following at 92 %, 76 %, 58 % and 20 % respectively in that order, this conform to findings by Mohamed *et al.* (2021), where *C. icipe* was

observed to have accepted younger instar stages of Fall armyworm larvae and also rejected older Fall armyworm larvae. There is also a report on *C. icipe* acceptance of *Spodoptera littoralis* and *S. exigua* second instar larvae for parasitism, with mean development time from egg to adult in 14 days under 25 ± 2˚C, 60–70% RH and a photoperiod of 12L: 12D laboratory conditions (Faiboe *et al*., 2017*)*. Fall armyworm larva stages at five days to 9 days; 3rd to 5th larva (Pitre and Hogg 1983), instar were not suitable for parasitism and therefore recorded lowest to no preference (parasitism) at 6 percent for five days and 0 percent for six days to nine days old larvae respectively. Although *C. icipe* differentially accepted newly emerged to 4 days Fall armyworm larvae for parasitism, several factors like Fall armyworm larvae mortality and adult parasitoid emergence plays crucial role in choosing an ideal Fall armyworm larva stage, were there is a substantial percentage of parasitism, adult parasitoid emergence and a lower Fall armyworm mortality. Amongst the preferred Fall armyworm larvae, one day old larvae (1st instar larva) had the highest ideal preference value of 0.47, with the highest parasitism, adult parasitoid emergence and the lowest Fall armyworm mortality at 94 percent parasitism level and 6 percent Fall armyworm mortality, followed by two days and day zero at an ideal preference value of 0.46 and 0.33 respectively The ideal preference stage is important in choosing the Fall armyworm stage that enhances the parasitoid population through reproduction and higher survival of parasitoid larvae until adult parasitoid emergence. One day and two days old Fall armyworm larvae are ideal for mass rearing of *C. icipe*. Similar observation was reported in *Cotesia marginiventris* (Cresson) which is also solitary is reported to prefer larva instar 1 for oviposition and later emerge out of 4th instar Fall armyworm larva (Cave, 1995).

Coccygidium luteum on the other hand recorded 62, 74, 82, 92, 98, 98, 86, 22, 2, and 0 percentage parasitism (parasitoid larva emergence) and 90.5, 87.2, 95.3, 97.8, 96.0, 100, 100, 100, 20, and 0.0 percentage adult parasitoid emergence out of the parasitoid larvae in newly emerged Fall armyworm larva (1st instar larva) to nine days old Fall armyworm larvae respectively. Mortality levels varied in newly emerged Fall armyworm larva (day 0), one day, two days, three days, four days, five days, six days, seven days, eight days, and nine days old Fall armyworm larvae, as they recorded 38, 26, 16, 8, 2, 2, 0, 0, 0, and 0 percentage mortality respectively. There was significant difference between Fall armyworm larva stages in all test parameters. According to the results obtained on percentage parasitism, *C. luteum* in the no-choice test showed a range of Fall armyworm larva stage preference from newly emerged (early 1st instar) to seven days old larva (early 4th instar) with only 2 percent parasitism in eight days old larvae (late 4th instar). The highest preference was recorded in four and five days old larvae (2nd and early 3rd instar) at 98 percentage parasitism, with three days, six days, two days, one day, day zero and seven days old Fall armyworm larva following at 92 %, 86 %, 82 %, 74 %, 62 % and 22 %, respectively in that order, which is in congruence with Agboyi *et al.*, (2020), where female *C. luteum* wasp was reported to have parasitized (accepted) a day-old first instar Fall armyworm larva. Fall armyworm larva stages at eight days to nine days (late 4th instar to early 5th instar) were not suitable for parasitism and also recorded lowest to no preference (parasitism) rates at 2 and 0 percent, respectively. Percentage mortality and adult parasitoid emergence also affected the choice of an ideal Fall armyworm larva stage. Amongst the preferred Fall armyworm larvae stages, four and five days old larvae (2nd and early 3rd instar) had the highest ideal preference value of 0.50 and 0.49, with the

highest parasitism (98 %), adult parasitoid emergence (96 and 100 % respectively), and Fall armyworm mortality at 2 for both. Although Three days old larva (early 2nd instar) recorded a higher percentage parasitism of 92 % than that of six days (3rd instar) old larva at 86 %, six days Fall armyworm larvae ranked 3rd in ideal preference level of 0.46, as it recorded 100 % adult parasitoid emergence and 0 % Fall armyworm mortality as compared to three days old larva which recorded 97.7 % adult parasitoid emergence and 8 % mortality and ideal preference level of 0.44. Four and five days old (2nd and early 3rd instar) Fall armyworm larvae are ideal for mass rearing of *C. luteum* followed by three days old larva.

Preference observations for *C. icipe* and *C. luteum* changed in the choice test as parasitism decreased significantly ($P < 0.001$) in the preferred larva stages in no–choice test as compared to the choice test. In *C. icipe* choice test, newly emerged, one day, two days, three days and four days old Fall armyworm larva (1st to 2nd instar) recorded 20, 20, 20, 60, and 20 percent parasitism respectively as against 76, 94, 92, 58, and 20 % recorded in the no-choice test. Five days old larvae (early 3rd instar) were not parasitized (accepted). Percentage adult parasitoid emergence was also affected as adult parasitoid emergence decreased at 20 percent for Fall armyworm day zero to three days old larvae (1st to early 2nd instar), and 40 percent for four days old larvae as against 82.7, 93.8, 100, and 97.2 percent for day zero to four days old (1st to late 2nd instar) Fall armyworm larvae respectively in no-choice test. Percentage mortality increased in the newly emerged to four days old (1st to 2nd instar) of Fall armyworm larvae at 80 percent for day zero to two days old, and 40 percent for four and five days larvae respectively with significant difference between Fall armyworm stages ($P < 0.001$). All the remaining Fall armyworm

larva stages recorded zero percentage mortality except five days old (early 3rd instar) larvae which recorded 20 percent mortality, which can be attributed to multiple probing of the early Fall armyworm stages (day zero to 2 days old) observed during the study. *Coccygidium luteum* recorded an average percentage parasitism of 20, 40, 40, 60, 80, 80, and 40 % for day zero to six days old (1st to 3rd instar) larvae respectively, and 0 % for seven days to nine days old (early 4th to 5th instar) Fall armyworm larvae in the choice test. The no-choice values were 62, 74, 82, 92, 98, 98, 86, 22, 2, and 0 % for day zero to nine days old Fall armyworm larvae respectively. The trend in parasitism values from day zero to nine days old (1st to early 5th instar) larvae was similar but with a lower percentage parasitism as compared to the no-choice test. This could be attributed to the increased number of female parasitoid probing in preferred larvae, as Fall armyworm numbers were lesser as compared to the choice test, resulting in a higher mortality. Percentage Fall armyworm larva mortality in the choice test was 80, 60, 60, 40, 20, 20, 20, 0, 0, and 0% as against 38, 26, 16, 8, 2, 2, 0, 0, 0, and 0% for day zero to nine days old (1st to early 5th instar) larvae, respectively in no-choice test. Parasitism mortality has been recorded in some Braconids, where endoparasitoid injects venom proteins or a virus during oviposition, in order to modify the behavior and damage the immune system of the host (Sanap *et al*., 2016). Percentage adult parasitoid emergence was 20, 40, 40, 20, 80, 80, 40, 0, 0, and 0 % in the choice test as against 90.5, 87.2, 95.3, 97.8, 96.0, 100, 100, 100, 20, and 0.0 % in day zero to nine days old (1st to early 5th instar) Fall armyworm larvae, respectively in no-choice test. The drop in adult parasitoid emergence can be attributed to the multiple parasitism and attack on Fall armyworm larvae, were aggression and struggling of Fall armyworm during oviposition may cause an increase in

dopamine level in Fall armyworm, which plays an important role in the retardation of larval growth and lead to death (Yamanaka *et al*., 1996; Fang *et al*., 2011).

The parasitism behaviour of parasitoids and response in Fall armyworm was also studied in choice and no-choice test. In the no-choice test, *C. luteum* interaction with Fall armyworm larvae during parasitism, behaviours such as parasitoid attack and no defense or physical aggression (AND) was expressed in day zero to three days old (1st to early 2nd instar) Fall armyworm larvae representing 100% of behavior expressed. Four to six days old (2nd to 3rd instar) Fall armyworm larva, expressed 88, 30, and 4 % behaviour response respectively, while seven days to nine days old (early 4th to 5th instar) larva expressed 0 % with significant difference between Fall armyworm larva stages ($P < 0.001$). *C. icipe* on the other hand recorded 100 % behaviour expression in newly emerged (day 0) and one day old (early 1st instar) Fall armyworm larvae, while two, three, and four days old (late 1st to 2nd instar) larva recorded 84, 58, and 4 %, respectively. Five, six, seven, eight and nine (9) days old (3rd, 4th and 5th instar) larva on the other hand recorded zero (0) % attack and no defense. This implies Fall armyworm stages five to nine days old (early 3rd to 5th instar) were not attacked by *C. icipe* as they express high level of physical aggression defense. Attempted parasitoid probing and defensive (aggression) behaviour in Fall armyworm larva (AD) was observed in four, five, six, seven, eight, and nine days old (2nd to 5th instar) larvae at 12, 70, 98, 88, 62, and 18 %, respectively, while day zero to three days old (1st to early 2nd instar) Fall armyworm larvae recorded 0 % of the defensive behaviour (aggression) during parasitism with significant difference between Fall armyworm age groups ($P < 0.001$), while *C. icipe* on the other hand recorded zero (0) % attack but defensive (AD) behaviour in newly emerged (early 1st instar) and one day old

(1st instar) Fall armyworm larva, where parasitoids attacked and probed these larva stages with no defense or aggression expressed, while Fall armyworm larva stages two to nine days old (late 1st to 5th instar) recorded 10, 24, 74, 58,40, 16, 4, and 4 % attack but defensive behavior (AD), respectively with a significant difference between Fall armyworm ages ($P < 0.001$). This implies older larvae express much defensive or aggressive behaviour during parasitoid attack. No parasitism attempt (NA) behaviour expression was 2, 12, 38, and 82 % in six to nine days old (late 3rd to 5th instar) Fall armyworm larvae, respectively and 0 % in day zero to five days old (1st to early 3rd instar) larvae respectively, with a significant difference between Fall armyworm larva age groups ($P < 0.001$). *C. icipe* on the other hand recorded 0 % no parasitism attempt behaviour expression in parasitism interaction with newly emerged (early 1st instar) and one day old (1st instar) Fall armyworm larvae, which implies day zero and one day old larvae are readily preferred for attack by *C. icipe* (parasitism), while two days to nine days old (late 1st instar to early 5th instar) Fall armyworm larva recorded 6, 18, 22, 42, 60, 84, 96, and 96 % , respectively in no parasitism attempt (NA) with significant difference between stages (ages) of Fall armyworm ($P < 0.001$). This implies as Fall armyworm get older attacked or accepted by *C. icipe* decreases, with the lowest non attempt parasitoid behaviour expression occurring in five days old (early 3rd instar) Fall armyworm larvae down to nine days old (early 5th instar) larvae respectively.

The above behaviour expressed in all the Fall armyworm age stages justifies why parasitoids attack or accept host species relative to their sizes, where larger parasitoids attacks older host stages and relatively smaller parasitoids attack younger host stages due

to an increase level of physical aggression as larvae (host) age increases (Blackburn, 1991a).

Behaviour expressions in the choice test was not different from the no choice test except in the choice test to parasitism attempt (NA) was made eight and nine days old Fall armyworm larvae by *C. luteum* parasitoid, while the no-choice test recorded an increase in attack but defensive behaviour (physical aggression) in five to eight days Fall armyworm larvae. *C. icipe* on the other hand recorded an increase in no parasitism attempt behaviour (NA) expression from four to nine days old larvae in the no-choice test, and an increase in parasitism attempt and aggressive defense behaviour (AD) in four to nine days old larvae as compared to the choice test which was lower at four to six days old larvae and 100 % no parasitism attempt (NA) in seven to nine days old Fall armyworm larvae. In this regard is important to state that the presence or absence of a preferred host affects the physical behaviour expressions of parasitoids, as demonstrated by Rosenheim (1996) where the number of suitable host that are encountered, and behavioural manipulation of the oviposition rate affect parasitoid behavior. Comparing these behaviour expression levels to the percentage parasitism in the Fall armyworm stages, in *C. luteum*, newly emerged to three days and four days old (early 2^{nd} to late 2^{nd} instar) Fall armyworm larvae expressed 100 % and 88 % attack with no physical defense through aggression respectively, and 62, 74, 82, and 92 % parasitism for newly emerged to three days old larvae respectively, and 98 % for four days old larvae. This demonstrates a diminished host phenol-oxidase activity and lack of melanotic encapsulation that function in cuticular melanization and sclerotization against invaders in insects, as reported in parasitoid-infected Lepidoptera (Cerenius & Soderhall, 2004;

Schnitger *et al.*, 2007). Successful wasp species introduce virulence factors into the host that suppress hemocyte-mediated encapsulation causing higher mortality in Fall armyworm larvae, and tend to decrease as host age increases, as described by Beck *et al.*, (2000), Beckage *et al.*, (1990), and Strand & Noda, (1991). Five to seven days old Fall armyworm larvae expressed physical aggressive behaviour which was countered by the intense strength and aggression of the *C. luteum* parasitoid, as they were observed to have carries up to 4 days old larvae about with the ovipositor inserted in the larvae. Eight to nine days old larvae expressed physical aggression defenses to a large extent against *C. luteum*, where percentage parasitism was 2 and 0 %, respectively. *C. icipe* expressed similar parasitism behaviour and Fall armyworm response at different percentage levels, where only day zero and one day old larvae recorded a 100% parasitoid attack and no defense behaviour expression. Two and three days old larvae recorded 84 and 58 % attack and no defense behaviour, with parasitism percentage of 92 and 58 %, respectively. While four to nine days old Fall armyworm larvae recorded higher levels of attempted parasitism and defense in larvae (AD), and no parasitism attempt behaviour (NA) with lower to no corresponding percentage parasitism levels (6 % for five days and 0 % for six to nine days old larvae).

The general findings for *C. icipe* and *C. luteum* preference agree with the principle by Blackburn (1991a), where parasitoid size is known to have a positive correlation to host preference size. Larger parasitoids always tend to have parasitism preference for larger host and vice versa. Fall armyworm larva mortality after parasitism shows an inverse proportionality to Fall armyworm stage, where mortality decreases with increase in age. The Fall armyworm larvae preference of *C. icipe* and *C. luteum* overlaps. *C. icipe* Fall

armyworm larvae stage preference Fall within the wider range of preference for *C. luteum* but with different ideal Fall armyworm preference stage to ensure population increase and survival.

5.3 Parasitism potential

Parasitism potential of endoparasitoids is an important aspect in considering parasitoid species for an effective biological pest control. *Coccygidium luteum* and *Cotesia icipe* parasitism potentials were tested on their respective preferred Fall armyworm larva stages from the first day of adult parasitoid emergence to day five. *C. luteum* recorded 0, 41, 40, 33, and 35 daily parasitism out of 50 Fall armyworm larvae, representing 0, 82, 79, 66, and 71 % parasitism on day one to day five, respectively. *Cotesia icipe* on the other hand recorded 10, 31, 17, 29, and 33, representing 19, 61, 34, 46, and 65 % parasitism, respectively. Parasitism means for all the day varied significantly for both parasitoid species ($P < 0.001$). There was no parasitism in *C. luteum* on the first day of Fall armyworm larva introduction, it however increased sharply on day two and continued to drop steadily from day three to four, and finally began increasing on day five. This kind of parasitism trend depicts extreme synovigenic egg maturation with no egg maturation at emergence, where parasitoid female eggs continue developing throughout their reproductive life as described by Jervis and Copland (1996). Flanders (1950) reported Family: Ichneumonidae, Braconidae as typical parasitoid examples. According to Jervis *et al*., (2001), the vast majority of species within the Parasitica and the Aculeata families of the order Hymenoptera have been found to be synovigenic, while only few species are unambiguously pro-ovigenic, parasitoids that have either all or almost all of their eggs

mature prior to the start of oviposition. It is likely male *C. luteum* species are moderately synspermatogenic, where males emerge with some spermatozoids and can mate shortly after emergence as observed in in *C. luteum* mating shortly after emergence on day one, but they have the capacity to produce spermatozoids throughout their life (Boivin *et al*., 2005). Unlike the case in *Bracon hebetor* (Hymenoptera: Braconidae) males which do not mate until several hours after emergence, although spermatozoids are present in the seminal vesicles (Gerling & Rotary 1974; Ode *et al*., 1996). The moderately synspermatogenic nature of male *C. luteum* observed in this study is supported in findings reported by Ramadan *et al*., (1991) and also confirmed by Quimio and Walter (2000), where parasitoids in the family Braconidae are reported to Fall within moderately synspermatogenic and synspermatogenic. *C. icipe* on the other hand recorded a small number of parasitoid (n= 10) on first day of emergence, but almost all the emerged parasitoids were male with only one female. Parasitism also increase largely on the second and decreased steadily from day three to four, and finally began increasing on day five as observed *in C. luteum*. In the case of *C. icipe*, the study recorded the smallest parasitism number in the first day of emergence, however Mohamed *et al*. (2021) reported a substantial number of eggs matured in newly emerged day 0 dissected female *C. icipe* wasp. The contrast in the parasitism level recorded in the study against the reported mature eggs in dissected *C. icipe* is due to the difference in the two biological events, as ovigeny is not same as parasitism. The number of mature eggs in a female parasitoid does not necessarily correspond to parasitism, as the number of eggs that a female parasitoid deposits during her lifetime is determined by interactions among three processes: the number of suitable hosts that are encountered, the number of eggs that are

matured throughout the female's life span, and behavioral manipulation of the oviposition rate (Rosenheim, 1996). There has been a report on egg resorption in some parasitoids that produce yolk-deficient (hydropic) eggs than the production of yolk-rich (anhydropic) eggs as a result of a higher proportion of eggs mature upon female emergence like the case of *C. icipe*, as reported by Jervis and Kidd (1986). This reproductive manipulative strategy could cause a reduction in the total number of parasitism. The daily parasitism level recorded in the study to a larger extent corresponds to *C. icipe* ovigeny index of 0.53 reported by Mohamed *et al.,* (2021). This implies a weak pro-ovigeny as smaller number of parasitism was recorded and also the general case for Braconidae where ovigeny index ranged from 0 to 0.74 which indicates extreme synovigeny to weak pro-ovigeny (Potting *et al*. 1997; Lim 1982). *Coccygidium luteum* recorded the highest total parasitism of 148, while *C. icipe* recorded 112 out of 250 Fall armyworm larvae, which represents 59.12 % and 44.72 %, respectively, but there was no significant difference in total parasitism between both parasitoid species (P = 0.507). The lower *C. icipe* parasitism level relative to that of *C. luteum* can be attributed to the higher percentage mortality recorded in *C. icipe* parasitized Fall armyworm larvae, as mortality had different effect on Fall armyworm larva stages from the preference test. Mortality increases as Fall armyworm age decreases. The daily average percentage mortality for *C. luteum* was 4.2, 3.3, 2, 1.6, and 3 %, as compared to 23.6, 4.4, 1.8, 5.6, and 5.6 % in *C. icipe* for day one to five, respectively. Percentage mortality was generally higher on day one at 4.5 % and 23.6 %, which later dropped on day two and three, and finial increased from day four to five but below the level of day one for *C. luteum* and *C. icipe*, respectively. Though there was a significant difference in the means of daily percentage

mortality of *C. icipe* ($P < 0.001$), *C. luteum* on the other hand recorded mean difference with a minute significance level ($P = 0.046$). Mortality was a main cause factor in a lower parasitism recorded in *C. icipe*. According the study observations, *C. luteum* attacks and manipulates Fall armyworm host faster as they have a relatively larger body size. This attribute gives *C. luteum* an added advantage in percentage parasitism as parasitism time was same for both parasitoid species. There was a negative correlation of daily percentage parasitism to percentage mortality, as *C. icipe* and *C. luteum* recorded the lowest parasitism on day one but highest mortality on day one and the lowest mortality thereafter when percentage parasitism increased sharply. *Coccygidium luteum* recorded 0, 25, 25, 26, and 22 males, which represents 0, 61, 63, 80 and 80 %, and 0, 16, 15, 7, and 6 females, representing 0, 39, 37, 20 and 20 % for day one to day five, respectively. *Cotesia icipe* recorded 9, 12, 8, 10, and 12 males, representing 94, 38, 48, 43 and 36 %, and 1, 19, 9, 13, and 21 females representing 6, 62, 52, 57 and 64 % for day one to day five, respectively. Although *C. icipe* recorded the lowest percentage parasitism in the first day of parasitoid emergence, all the parasitoids that emerged were males except one female. Female number increase twice as that of males in day two. Male and female sex ration almost became the same on day three with a slightly higher number in favor of females. There was a steady increase in female number on the fourth day and reach double the number of males in day five, as recorded in day two. The case was different in *C. luteum* as day one of adult parasitoid emergence recorded no parasitism. Day two however recorded the highest number of parasitism, followed by day three, with lower female ratio of almost 1 to 2 in favor of males. Female number decreased by half on day four and five, with male numbers remaining almost the same at a ratio of 1 female to 3

males. These observations may imply male *C. luteum* parasitoids are prospermatogenic species, where they have all their spermatozoids mature at emergence and do not produce more spermatozoids later in life as demonstrated by Wilkes (1966). Spermatogeny of the parasitoids should be studied to add up to knowledge of parasitoid behaviors. The total average male and female *C. luteum* parasitoids recorded in five days were 98 and 42, representing 70 and 30 % for male and female, respectively, while *C. icipe* recorded a total of 50 and 62, representing 45 and 55 % male and females, respectively. There was a little higher female to male ratio in *C. icipe* at 1 male to 1.3 females which is approximated to 1 male♂: 1 female♀ ration as compared to *C. luteum* which had a double male to female ratio of 2.3 males to 1 female (2 M♂: 1 F♀).

5.4 Interaction between parasitoids and Fall armyworm

Parasitism interaction between *C. luteum* and *C. icipe* in Fall armyworm was studied by allowing both parasitoids the chance to oviposit in the same Fall armyworm larva, while the larva was followed daily to observe and record the total number of each parasitoid species that emerged. The test was carried out in a sequential introduction of the parasitoid species, where *C. luteum* is introduced first to the Fall armyworm larvae and latter same larvae introduced to *C. icipe*, and vice versa. There was also a concurrent introduction of both parasitoids to Fall armyworm larvae. Generally both species accepted already parasitized larvae by the other parasitoid species. In the first introduction of *C. luteum* to Fall armyworm larvae before later introduction to *C. icipe*, a total of 36 and 44 which represents 24 % and 29.4 % for *C. luteum* and *C. icipe*, respectively emerged from Fall armyworm larva, while the first introduction of *C. icipe*

to Fall armyworm larvae before later introduction to *C. luteum* recorded a total of 35 and 42, representing 23.4 % and 28 % for *C. luteum* and *C. icipe*, respectively. Although the number of *C. icipe* parasitoid species recorded was a little higher as compared to *C. luteum* in both parasitoid introduction sequence, however there was no significant difference between *C. icipe* and *C. luteum* emergence in both *C. icipe* first and *C. luteum* first sequential introduction. *C. icipe* had a smaller body size as compared to *C. luteum* and gives it an advantage in interspecific competition in both sequence of parasitoids introduction, were smaller body size correlates to shorter development time (Blackburn, 1991a; Stearns, 1992), this was also evident in the faster development of *C. icipe* larvae before emerging out of the Fall armyworm larva and also the shorter development time from parasitoid larvae to pupae, with eyes and pseudo legs already forming (figure: 4.1.6) and finally a shorter pupae to adult development time as compared to *C. luteum* with a longer development time. Early female parasitoid introduction or exposure to a host usually causes a longer period of competition between parasitized and healthy larvae, where earlier parasitoid introduction or arrival had been reported to cause a competitive advantage to the weaker or smaller species. They out-compete the stronger ones if the time advantage was longer (Cameron *et al*., 2005; Octavio *et al*., 2018). This phenomenon also supports the slightly higher number of *C. icipe* recorded in the first introduction of *C. luteum* to Fall armyworm larvae before *C. icipe* in the sequential parasitoid introduction test.

Concurrent introduction of parasitoids to Fall armyworm larvae on the other hand was for a shorter duration (20 minutes) as compared to sequential introduction test (30 minutes). *C. luteum* recorded a higher parasitism number of 46, representing 30.7 % as compared to

19 in *C. icipe*, which represents 12.7 % parasitism. The larger size of *C. luteum* gives it an added advantage again as it manipulates the Fall armyworm larvae with ease, as it was observed to have carried Fall armyworm larva with the ovipositor after probing. Such characteristic reduces time wasted by the parasitoid to counteract host physical defense through aggression and contributes to a relatively faster parasitism as compared to *C. icipe*. A longer parasitoid- host exposure time could give an added advantage to smaller sized insect parasitoids as reported by Octavio *et al*., (2018). The drop in *C. icipe* parasitism level in the concurrent test could also be attributed to a lesser time of exposure (20 minutes) as compared to sequential parasitoid introduction.

There is competition between *C. icipe* and *C. luteum* as Fall armyworm parasitism preference overlap in both parasitoid species and both parasitoids accept Fall armyworm larvae already parasitized by the other species. The competition between both parasitoid species caused a reduction in the total number of parasitism as compared to parasitism in only one parasitoid species introduced to Fall armyworm larva. Intrinsic interspecific competition mechanisms have been reported to exist amongst Braconidae wasps, parasitizing same host species (Cancino *et al*., 2014; Murillo et al., 2016; Paranhos *et al*., 2013) and parasitoid species whose host preference overlaps (Cusumano *et al*., 2012) . However the competition between the two parasitoids does not favour one species over the other, as no significant difference was found in the total parasitism of *C. luteum* and *C. icipe* in all the first introduction of either parasitoids to Fall armyworm before the other (sequential) (P = 0.357 and 0.298 for *C. icipe* 1st and *C. luteum* 1st, respectively), except the concurrent introduction of parasitoids to Fall armyworm which recorded a significant parasitism difference in favour of *C. luteum* (P < 0.001). According to Feng *et*

al., (2015), interspecific competition can lead to successful long term coexistence of parasitoids who exploits the same host but partially different stage preference as in the case of *C. luteum* and *C. icipe*.

Mortality generally increased in the interaction test as parasitoids exploited the same host for parasitism. Total percentage mortality was highest in *C. icipe* first, *C. luteum* first and concurrent introduction of both parasitoids to Fall armyworm larvae at 45.4 %, 46.0 %, and 44.3 %, respectively. Mortality was however lowest in the control where no Fall armyworm larva was exposed for parasitism, followed by only *C. icipe* and only *C. luteum* at 2.0 %, 4.7 % and 17.4 %, respectively. Mortality increased significantly in all the sequence of parasitoid introduction to Fall armyworm as compared to only one parasitoid introduction to Fall armyworm larvae and the control without parasitism, as a result of competition between the two parasitoid species. Percentage mortality difference amongst the first introduction of parasitoids concurrently and sequentially to Fall armyworm was however not significant as they all recorded mortality between 44 to 46 percent. According to Haeselbarth (1979), multiple parasitism may cause several eggs in a clutch within the host, which may lead to early larva mortality as the parasitoid larva feeds within the gut lumen of the host. This was also observed by Cameron and Benton (2004) as clutch sizes from multiple parasitism causes an increase in mortality. The competition between *C. luteum* and *C. icipe* for the same Fall armyworm host resource could affect the establishment and increase of these parasitoids population, as both intrinsic and extrinsic interspecific competition mechanisms has been reported to exist amongst Braconidae wasps, parasitizing same host species (Cancino *et al*., 2014; Murillo *et al.,* 2016; Paranhos *et al*., 2013). The general percentage parasitism and increase in

Fall armyworm mortality in the competition tests indicates a synergism effect of both parasitoids on Fall armyworm control. Although the competition between these parasitoid species caused a drop in the percentage parasitism of both species, the total percentage parasitism in Fall armyworm larvae introduced in the competition test was above fifty percent which was not too far apart from the total parasitism of only one parasitoid without competition. In these regard the competition may also serve as a population check of both species, and periodic augmentation could be done to revamp their populations. The competition however caused an increased in percentage mortality in the interaction between *C. luteum* and *C. icipe* as compared to the mortality effect of only one parasitoid species attack on Fall armyworm and the control. The mortality of Fall armyworms attacked by both parasitoids occurred faster at a maximum of three days after exposure to both parasitoids.

The study has indicated that the differences in the population of *C. icipe* and *C. luteum* in East African countries; Tanzania and Kenya and West African countries; Ghana, Benin, and Togo may not be as a result of competition between both species in their respective sub-regions, as it was a subject of concern in choosing one or both parasitoid for biocontrol in Ghana. The percentage parasitism of both species was not significantly different in the competition test when *C. icipe* was first introduction to the Fall armyworm larvae before *C. luteum* (P = 0.357) and the reverse (P = 0.298). Concurrent introduction however took an exception, with a significant difference in percentage parasitism in favour of *C. luteum*. The difference in the percentage parasitism of these species could be attributed to the sampling methods and Fall armyworm stages collected. The comparism could be more substantial or appropriate if equal numbers of preferred

Fall armyworm stages were sampled in both regions. This was not the case in Agboyi *et al.,* (2020) where Fall armyworm were grouped according to ranges; young, medium and old. Sisay *et al.,* (2018) on the other hand sampled Fall armyworm larvae according to instars with different preference sample sizes. There is a likelihood of recording more population density of *C. luteum* due to its larger host range as compared to *C. icipe*.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Competition exists between *Coccygidium luteum* and *Cotesia icipe* within Fall armyworm larva during parasitism and their preference for Fall armyworm larval stage overlaps. The competition however works in synergy to cause a significant increase in Fall armyworm larval percentage mortality. A substantial number of total percentage parasitism was recorded which keeps a sustainable parasitoid population of both species. The generation time of both parasitoids was not above thirty days and below fourteen days interval, with multiple generations per year which is in synchrony with the generation time and voltinism of the Fall armyworm. Both *C. luteum* and *C. icipe* are good candidates for an effective Fall armyworm biocontrol programme as they work synergistically in controlling the Fall armyworm pest through a significant increase in Fall armyworm percentage mortality.

6.2 Recommendations

Spermatogeny test must be carried out in both male parasitoid species for better understanding of their sperm production as spermatogeny also affects the reproductive capacity of females.

It is also recommended that mating behaviour should be studied in both species as some males and females of certain species may either mate once or throughout their life span. Knowledge of their mating behaviour would enhance a better drafting of methods in

parasitoid reproduction studies, as males who mate once in their life time would definitely affect the reproductive capacity of female if females are paired with one male in a parasitism test over a period of time.

Four and five days old (2^{nd} and early 3^{rd} instar), and one days old (1^{st} instar) Fall armyworm larva are ideal and recommended for laboratory rearing of *C. luteum* and *C. icipe*, respectively, as percentage parasitism and adult parasitoid emergence are higher, with lower percentage Fall armyworm mortality after probing.

It is further recommended that, alternative hosts should be considered for mass rearing of these Fall armyworm larval parasitoids as cannibalism starts right after the second larva instar and would affect successful adult parasitoid emergence.

Finally, it is recommended that the true abundance, distribution and population density of these species should be determined in a survey, with the same number of preferred Fall armyworm larval stages of both parasitoid species.

REFERENCES

Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N.; Day, R.; Early, R.; Godwin, J. (2017). . Fall Armyworm: Impacts and Implications for Africa; UKAid: Exeter, UK; CABI: Exeter, UK,.

Agboyi, L.K., Goergen, G., Beseh, P., Mensah, S. A., Clottey, V. A., Glikpo, R., & Rwomushana, I. (2020). Parasitoid complex of Fall armyworm, *Spodoptera frugiperda*, in Ghana and Benin. *Insects*, 11(2): 68.

Agboyi, L.K., Mensah, S. A., Clottey, V. A., Beseh, P., Glikpo, R., Rwomushana, I., & Kenis, M. (2019). Evidence of Leaf Consumption Rate Decrease in Fall Armyworm, *Spodoptera frugiperda*, Larvae Parasitized by *Coccygudium lureum. Insects*, 10(11): 410.

Agric. Research Council, South Africa (2017). FAWarned. Retrieved March 2021. https://www.arc.agric.za/arc-ppri/FAWarned/Pages/Identification-characteristics.aspx.

Alyokhin, A. V., & Messing, R. H. (2003). Parasitism of Hawaiian non-frugivorous fruit flies (Diptera: Tephritidae) by an exotic parasitoid, *Eurytoma tephritidis* Fullaway (Hymenoptera: Eurytomidae). *Hawaiian Entomological Society* 36: 29-37.

Ashley, T. R. (1986). Geographical distributions and parasitization levels for parasitoids of the Fall armyworm, *Spodoptera frugiperda*. *Florida Entomologist*, 69(3): 516-524.

Ashley T. R, Wiseman BR, Davis FM, Andrews KL. (1989). The Fall armyworm: a bibliography. *Florida Entomologist*, 72: 152-202.

Beck, M., Theopold, U., & Schmidt, O., (2000). Evidence for serine protease inhibitor activity in the ovarian calyx fluid of the endoparasitoid *Venturia canescens*. *Journal of Insect Physiology*. 46: 1275–1283.

Beckage, N., Metcalf, J., Nesbit, D., Schleifer, K., Zetlan, S.R., De Buron, I. (1990). Host hemolymph monophenoloxidase activity in parasitized *Manduca sexta* larvae and evidence for inhibition by wasp polydnavirus. *Insect Biochemistry.* 20: 285–294.

Beckage, N.E.; Riddiford, L.M. (1982). Effects of parasitism by *Apanteles congregatus* on the endocrine physiology of the tobacco hornworm, *Manduca sexta*. *General and Comparative Endocrinology*. 47(3): 308–322.

Beckage, N.E.; Riddiford, L.M. (1978), Developmental interactions between the tobacco hornworm Manduca sexta and its braconid parasite *Apanteles congregates* L. *Entomologia Experimentalis et Applicata*, 23(2):139–151.

Blackburn, T. M. (1991). Evidence for a fast-slow'continuum of life-history traits among parasitoid Hymenoptera. *Functional Ecology*, 5(1): 65-74.

Bidla, G., Dushay, M.S., Theopold, U., 2007. Crystal cell rupture after injury in *Drosophila* requires the JNK pathway, small GTPases and the TNF homolog Eiger. *Journal of cell science, 120(7)*: 1209-1215.

Boisduval, J.B. (1833). *Hesperia borbonica* Boisduval, 1833. *Nouvelles Annales du Meseum d'Histoire Naturalle*, 2: 213.

Boivin, G., Jacob, S., & Damiens, D. (2005). Spermatogeny as a life-history index in parasitoid wasps. *Oecologia*, 143(2): 198-202.

Boots, M., & Norman, R. (2000). Sublethal infection and the population dynamics of host–microparasite interactions. *Journal of Animal Ecology*, 69(3): 517-524.

Boxall, A. B., Sinclair, C. J., Fenner, K., Kolpin, D., & Maund, S. J. (2004). Peer reviewed: when synthetic chemicals degrade in the environment. *Environmental Science & Technology*, 38(19): 368-375.

Brammall, R. A., & Higgins, V. J. (1988). The effect of glyphosate on resistance of tomato to Fusarium crown and root rot disease and on the formation of host structural defensive barriers. *Canadian Journal of Botany*, 66(8): 1547-1555.

Brodeur, J., Geervliet, J. B., & Vet, L. E. (1998). Effects of *Pieris* host species on life history parameters in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species). *Entomologia Experimentalis et Applicata*, 86(2): 145-152.

Cabello, T., Gámez, M., Torres, A., & Garay, J. (2011). Possible effects of inter-specific competition on the coexistence of two parasitoid species: *Trichogramma brassicae* Bezdenko and *Chelonus oculator* (F.) (Hymenoptera: Trichogrammatidae, Braconidae). *Community Ecology*, 12(1): 78-88.

Cameron, P. (1904). On some new genera and species of Hymenoptera. *American Entomological Society*, 30(4): 251-267.

Cameron, T. C., & Benton, T. G. (2004). Stage-structured harvesting and its effects: an empirical investigation using soil mites. *Journal of Animal Ecology*, 73(5): 996-1006.

Cameron, T. C., Wearing, H. J., Rohani, P. and Sait, S. M. (2005). A koinobiont parasitoid mediates competition and generates additive mortality in healthy host populations. *Oikos*, 110: 620-628.

Cancino, J., Montoya, P., Barrera, J. F., Aluja, M., & Liedo, P. (2014). Parasitism by *Coptera haywardi* and *Diachasmimorpha longicaudata* on *Anastrepha* flies with different fruits under laboratory and field cage conditions. *BioControl*, 59: 287-295.

Caniço, A., Mexia, A., & Santos, L. (2020). First Report of Native Parasitoids of Fall Armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Mozambique. *Insects*, *11*(9): 615.

Capinera, J. L. (2002). Fall Armyworm, *Spodoptera frugiperda* (JE Smith)(Insecta: Lepidoptera: Noctuidae): EENY098/IN255, rev. 7/2000. *Edis*, *2002*(7).

Cave, R. D. (1995). Manual para el reconocimiento de parasitoides de plagas agrícolas en América Central. El Zamorano: Zamorano Academic Press.

Cerenius, L., Soderhall, K., (2004). The prophenoloxidase-activating system in invertebrates. *Immunological Reviews*, 198: 116–126.

Christensen, B.M., Li, J., Chen, C.-C., Nappi, A.J., 2005. Melanization immune responses in mosquito vectors. *Trends in parasitology*, *21*(4): 192-199.

Cock, M.J.W., Beseh, P.K., Buddie, A.G., Cafá, G. & Crozier, J. (2017). Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. *Scientific Reports* ,7(4103): 10 pp. doi:10.1038/s41598-017-04238-y.

Costamagna, A. C., & Landis, D. A. (2004). Effect of food resources on adult *Glyptapanteles militaris* and *Meteorus communis* (Hymenoptera: Braconidae), parasitoids of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). *Environmental Entomology*, 33(2): 128-137.

Cruz, I., Figueiredo, M. L. C., Oliveira, A. C., & Vasconcelos, C. A. (1999). Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. *International Journal of Pest Management*, 45(4): 293-296.

Cusumano, A., Peri, E., Bradleigh Vinson, S., & Colazza, S. (2012). Interspecific extrinsic and intrinsic competitive interactions in egg parasitoids. *BioControl*, 57: 719-734.

Day, R.; Abrahams, P.; Bateman, M.; Beale, T.; Clottey, V.; Cock, M.; Colmenarez, Y.; Corniani, N.; Early, R.; Godwin, J.; Gomez, J.; Gonzalez Moreno, P.; Murphy, S.T.; Oppong-Mensah, B.; Phiri, N.; Pratt, C.; Silvestri, S.; Witt, A. (2017). Fall armyworm: impacts and implications for Africa. *Outlooks Pest Management*, 28: 196-201. DOI: 10.1564/v28_oct_02.

De Gregorio, E., Han, S.J., Lee, W.J., Baek, M.J., Osaki, T., Kawabata, S.I., et al., (2002). An immune-responsive serpin regulates the melanization cascade in Drosophila. *Developmental cell*, *3*(4): 581-592.

Delaplane, K. S. (2001). *Varroa destructor*: revolution in the making. *Bee World*, 82(4), 157-159.

Dequech, S.; Camera, C.; Sturza, V.; Ribeiro, L.; Querino, R.; Poncio, S (2013). Population fluctuation of *Spodoptera frugiperda* eggs and natural parasitism by Trichogramma in maize. *Acta Scientiarum. Agronomy*, 35: 295–300.

Dias, P. (2019). Analysis of incentives and disincentives for cassava in Mozambique. *Gates Open Res*, *3*(642): 642.

Fang, Q.; Wang, F.; Gatehouse, J.A.; Gatehouse, A.M.R.; Chen, X.; Hu, C.; Ye, G.(2011) Venom of parasitoid, *Pteromalus puparum*, suppresses host, *Pieris rapae*, immune promotion by decreasing Host C-Type Lectin Gene Expression. PLoS One, 6(10): e26888.

Farahani, S., Talebi, A., Rakhshani, E.' Achterberg, C.; Sharkey, M (2014). A contribution to the knowledge of Agathidinae (Hymenoptera: Braconidae) from Iran with description of a new species. *Biologia*, 69: 228–235.

Feng, Y., Wratten, S., Sandhu, H., & Keller, M. (2015). Interspecific competition between two generalist parasitoids that attack the leafroller, *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research*, 105(4): 426-433.

Fernández-Triana, J. L., Janzen, D. H., Hallwachs, W., Whitfield, J. B., Smith, M. A., & Kula, R. (2014). Revision of the genus *Pseudapanteles* (Hymenoptera, Braconidae, Microgastrinae), with emphasis on the species in Area de Conservación Guanacaste, northwestern Costa Rica. *ZooKeys*, (446), 1.

Fiaboe, K. K., Fernández-Triana, J., Nyamu, F. W., & Agbodzavu, K. M. (2017). *Cotesia icipe* sp. n., a new Microgastrinae wasp (Hymenoptera: Braconidae) of importance in the biological control of Lepidopteran pests in Africa. *Journal of Hymenoptera Research*, 61: 49-64.

Flanders, S.E. (1950) Regulation of ovulation and egg disposal in the parasitic Hymenoptera. *Canadian Entomologist*, 82: 134–140.

Fletcher, A., Bill, D. J., Bill, S. J., Cliffe, I. A., Dover, G. M., Forster, E. A. & Reilly, Y. (1993). WAY100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. *European Journal of Pharmacology*, 237(2-3): 283-291.

Fritz, S., See, L., mccallum, I., You, L., Bun, A., Moltchanova, E., Duerauer, M., Albrecht, F., Schill, C., Perger, C., and Havlik, P. (2015). Mapping global cropland and field size. *Global Change Biology*, 21(5), 1980–1992. DOI: 10.1111/gcb.12838

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W. W., Emmerson, M., Morales, M. B., & Inchausti, P. (2010). Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*, 11(2); 97-105.

Gerling D, Rotary N., (1974) Structure and function of the seminal vesicles and the spermatheca in *Bracon hebetor* Say (Hymenoptera: Braconidae). *International Journal of Insect Morphology and Embryology*, 3:159–162.

Gilbert, F., & Jervis, M. (1998). Functional, evolutionary and ecological aspects of feeding-related mouthpart specializations in parasitoid flies. *Biological Journal of the Linnean Society*, 63(4); 495-535.

Godfray, H. C. J. (1994). *Parasitoids: behavioral and evolutionary ecology* (Vol. 67). Princeton University Press.

Godfray, H.C.J (2007). Parasitoids. *Encyclopedia of Biodiversity. Elsevier, Oxford*, 10: 1-12.

Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M. (2016). First report of outbreaks of the Fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. PLoS ONE, DOI: 10.137/journal.pone.0165632.

Graham, I. M. and Lambin, X. (2002). The impact of weasel predation on cyclic field-vole survival: the specialist predator hypothesis contradicted. *Journal of Animal Ecology*, 71: 946-956.

Gutiérrez-Moreno, R.; Mota-Sanchez, D.; Blanco, C.A.; Whalon, M.E.; Terán-Santofimio, H.; Rodriguez-Maciel, J.C.; DiFonzo, C. (2019). Field-evolved resistance of the Fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. *Journal of economic entomology*, 112(2): 792–802.

Haeselbarth, E. (1979). On the pupal parasites of the pine beauty (*Panolis flammea* [Schiff.]), the pine looper (*Bupalus piniarius* [L.]) and of *Boarmia bistortata* (Goeze) in Bavarian pine forest [Germany, FR]. *Zeitschrift fuer Angewandte Entomologie (*Germany, FR*)*.

Hailu, G.; Niassy, S.; Zeyaur, K.R.; Ochatum, N.; Subramanian, S (2018). Maize-Legume Intercropping and Push-Pull for Management of Fall Armyworm, Stemborers, and Striga in Uganda. *Agronomy Journal*, 110(6): 2513–2522.

Harrison, R.D.; Thierfelder, C.; Baudron, F.; Chinwada, P.; Midega, C.; Schaffner, U.; van den Berg, J (2019). Agro-ecological options for Fall armyworm (*Spodoptera frugiperda* JE Smith) management: Providing low-cost, smallholder friendly solutions to an invasive pest. *Journal of environmental management*, 243: 318–330.

Hay-Roe, M.M.; Meagher, R.L., Nagoshi, R.N., Newman, Y. (2016). Distributional patterns of Fall armyworm parasitoids in a corn field and a pasture field in Florida. *Biological Control*, 96: 48–56.

Heidari, H. (2012). Effect of defoliation intensity on maize yield, yield components and seed germination. *Life Science Journal*, 9(4): 1594-1598.

Hoballah, M. E., Degen, T., Bergvinson, D., Savidan, A., Tamo, C., & Turlings, T. C. (2004). Occurrence and direct control potential of parasitoids and predators of the Fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agricultural and Forest Entomology*, 6(1): 83-88.

Hruska, A.J (2019). Fall armyworm (*Spodoptera frugiperda*) management by smallholders. CAB Rev., 14: 1–11.

Huffaker, C. B. and Matsumoto, B. M. (1982). Group versus individual functional responses of *Venturia (Nemeritis) canescens* (Grav). *Researches on Population Ecology*, 24(2): 250/269.

Jeffries, M.J., Lawton, J.H., (1984). Enemy free space and the structure of ecological communities. *Biological Journal of the Linn*ean *Society* 23(4): 269–286. http://dx.doi.org/10.1111/j.1095- 8312.1984.tb00145.x.

Jervis. M.A. & Kidd, N.A.C. (1986) Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, 61(4): 395–434.

Jervis, M. A., Heimpel, G. E., Ferns, P. N., Harvey, J. A., & Kidd, N. A. (2001). Life-history strategies in parasitoid wasps: a analysis of 'ovigeny'. *Journal of Animal Ecology*, 70(3): 442-458.

Jervis, M.A. & Copland, M.J.W. (1996). The life cycle. *Insect Natural Enemies: practical approaches to their study and evaluation*, pp. 63–161. (eds. M. Jervis & N. Kidd), Chapman & Hall, London.

Jervis, M. A., Copland, M. J., & Harvey, J. A. (2005). The life-cycle. *Insects as Natural Enemies: A Practical Perspective*,: 73-165. https://doi.org/10.1007/978-1-4020-2625-6_2

Johnson SJ. (1987). Migration and the life history strategy of the Fall army worm in the western hemisphere. *Insect Science and its Application*, 8: 543-549.

Julio C. Fatoretto, Andrew P. Michel, Marcio C. Silva Filho, Nestor Silva. (2017); Adaptive Potential of Fall Armyworm (Lepidoptera: Noctuidae) Limits *Bt* Trait Durability in Brazil, *Journal of Integrated Pest Management*, 8 (1): 17.

Kansiime, K.M.; Mugambi, I.; Rwomushana, I.; Nunda, W.; Lamontagne-Godwin, J.; Rware, H.; Phiri, N.A.; Chipabika, G.; Ndlovu, M.; Day, R. (2019). Farmer perception of Fall armyworm (*Spodoptera frugiperda* J.E. Smith) and farm-level

management practices in Zambia. Pest Management Science, Online First. doi: 10.1002/ps.5504.

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, 10(10): 977-994.

Karlsson, D., & Ronquist, F. (2012). Skeletal morphology of *Opius dissitus* and *Biosteres carbonarius* (Hymenoptera: Braconidae), with a discussion of terminology. *PLoS One*, 7(4) : e32573.

Kenis, M., Du Plessis, H., Van den Berg, J., Ba, M. N., Goergen, G., Kwadjo, K. E., & Polaszek, A. (2019). *Telenomus remus*, a candidate parasitoid for the biological control of *Spodoptera frugiperda* in Africa, is already present on the continent. *Insects*, 10(4): 92. https://doi.org/10.3390/insects10040092.

Koffi, D.; Kyerematen, R.; Eziah, V.Y.; Agboka, K.; Adom, M.; Goergen, G.; Meagher, R.L., Jr (2020). Natural enemies of the Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Ghana. *Florida Entomologist*, 103(1): 85–90.

Kumela, T.; Simiyu, J.; Sisay, B.; Likhayo, P.; Mendesil, E.; Gohole, L.; Tefera, T (2018). Farmers' knowledge, perceptions, and management practices of the new invasive pest, Fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. *International journal of pest management*, 1–9. DOI: 10.1080/09670874.2017.1423129

Lah K (2011). Effects of pesticides on human health. In: Toxipedia. Available from *http://www.toxipedia.org/display/toxipedia/Effects+of+Pesticides+on+Human+Health.* Accessed March, 2021.

Lane, S. D., & Mills, N. J. (2003). Intraspecific competition and density dependence in an Ephestia kuehniella–Venturia canescens laboratory system. *Oikos*, *101*(3): 578-590.

Lezama-Gutiérrez, R.; Hamm, J.J.; Molina-Ochoa, J.; López-Edwards, M.; Pescador-Rubio, A.; González-Ramirez, M.; Styer, E.L. (2001). Occurrence of

entomopathogens of Spodoptera frugiperda (Lepidoptera: Noctuidae) in the Mexican states of Michoacan, Colima, Jalisco and Tamaulipas. *Florida Entomologist*, 84: 23–30.

Ligoxygakis, P., Pelte, N., Ji, C.Y., Leclerc, V., Duvic, B., Belvin, M., et al., (2002). A serpin mutant links Toll activation to melanization in the host defence of *Drosophila*. EMBO Journal. 21: 6330–6337.

Lim, G. S. (1986). Biological control of diamondback moth, pp. 159-171. In *Diamondback moth management: Proceedings of the first international workshop* . Shanhua, Taiwan: Asian Vegetable Research and Development Center.

Lim, G.S. (1982). The biology and effects of parasites on the diamond-back moth, *Plutella xylostella* (L.) Ph. D. Thesis, Univ. of London, 317 pp.

Liu, H., Jiravanichpaisal, P., Cerenius, L., Lee, B.L., Soderhall, I., Soderhall, K., (2007). Phenoloxidase is an important component of the defense against *Aeromonas hydrophila* Infection in a crustacean, *Pacifastacus leniusculus*. *Journal of Biological Chem*istry 282(8): 33593–33598.

Lockwood, J. A. (2000). Nontarget effects of biological control: what are we trying to miss? Pp. 15-30. In: P.A. Follett and J.J. Duan (eds.). Nontarget effects of biological control. Kluwer Academic Publishers. Boston, Massachusetts.

Macauley, H., & Ramadjita, T. (2015). Cereal crops: Rice, maize, millet, sorghum, wheat. https://ir.ucc.edu.gh/xmlui/bitstream/handle/123456789/4510/Cereal_Crops-_Rice__Maize__Millet__Sorghum__Wheat.pdf?sequence=1&isAllowed=y.

Mahmood, Isra, Sameen Ruqia Imadi, Kanwal Shazadi, Alvina Gul, and Khalid Rehman Hakeem (2016). "Effects of pesticides on environment in Plant, soil and microbes", *implications in crop science*, 1: 253-269.

Marenco RJ, Foster RE, Sanchez CA. (1992). Sweet corn response to Fall army worm damage during vegetative growth. *Journal of Economic Entomology*, 85: 1285-1292.

Margni, M., Rossier, D., Crettaz, P., & Jolliet, O. (2002). Life cycle impact assessment of pesticides on human health and ecosystems. Agriculture, Ecosystems & Environment, 93(1-3):379-392.

Mason, W. R. M. (1981). The polyphyletic nature of *Apanteles Foerster* (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. *The Memoirs of the Entomological Society of Canada*, 113(S115): 1-147.

Moghaddam M, Fernandez-Triana J, Ward D (2021). Microgastrinae Wasps of the World. *http://microgastrinae.myspecies.info/* [Accessed on 2021/ 05/12].

Mohamed SA, Wamalwa M, Obala F, Tonnang HEZ, Tefera T, Calatayud P-A, Subramanian, S. & Ekesi, S. (2021). A deadly encounter: Alien invasive *Spodoptera frugiperda* in Africa and indigenous natural enemy, *Cotesia icipe* (Hymenoptera: Braconidae). *PLoS ONE* 16(7): e0253122. https:// doi.org/10.1371/journal.pone.0253122.

Molina-Ochoa, J., Hamm, J. J., Lezama-Gutiérrez, R., López-Edwards, M., González-Ramírez, M., & Pescador-Rubio, A. (2001). A survey of Fall armyworm (Lepidoptera: Noctuidae) parasitoids in the Mexican states of Michoacán, Colima, Jalisco, and Tamaulipas. *Florida Entomologist*, 84(1): 31-36.

Montezano, D. G., Sosa-Gómez, D. R., Specht, A., Roque-Specht, V. F., Sousa-Silva, J. C., Paula-Moraes, S. D., ... & Hunt, T. E. (2018). Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *African Entomology*, 26(2): 286-300.

Murillo, F. D., Liedo, P., Nieto-López, M. G., Cabrera-Mireles, H., Barrera, J. F., & Montoya, P. (2016). First instar larvae morphology of Opiinae (Hymenoptera: Braconidae) parasitoids of Anastrepha (Diptera: Tephritidae) fruit flies. Implications for interspecific competition. *Arthropod Structure & Development*, 45(3): 294-300.

Nappi, A.J., Christensen, B.M., (2005). Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. *Insect Biochemistry and Molecular Biology* 35(5): 443–459.

Newman, R. M., Thompson, D. C., & Richman, D. B. (1998). Conservation strategies for the biological control of weeds, (pp. 371-396). In Conservation biological control Academic Press.

Octavio A. Bruzzone, Guillermo A. Logarzo, María B. Aguirre, Eduardo G. Virla (2018). Intra-host interspecific larval parasitoid competition solved using modelling and bayesian statistics. *Ecological Modelling*, 385: 114-123.

Ode PJ, Antolin MF, Strand MR (1996). Sex allocation and sexual asymmetries in intra-brood competition in the parasitic wasp, *Bracon hebetor*. *J. Anim Ecol*., 65: 690–700.

Pannuti L. E. R, Baldin E. L. L, Hunt T. E, & Paula-Moraes, S. V. (2016). On-plant larval movement and feeding behavior of Fall armyworm on reproductive corn stages. *Environmental Entomology*, 45: 192-200.

Paranhos, B. J., Sivinski, J., Stuhl, C., Holler, T., & Aluja, M. (2013). Intrinsic competition and competitor-free-space influence the coexistence of parasitoids (Hymenoptera: Braconidae: Opiinae) of Neotropical Tephritidae (Diptera). *Environmental Entomology*, 42(4): 717-723.

Pitre, HN, Hogg, DB. (1983). Development of the Fall armyworm on cotton, soybean and corn. *Journal of the Georgia Entomological Society*, 18: 187-194.

Potting, R. P. J., Overholt, W. A., Danso, F. O., & Takasu, K. (1997). Foraging behavior and life history of the stemborer parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae). *Journal of Insect Behaviour*, 10: 13-29.

Quicke, D. L. (1997). *Parasitic wasps*. Chapman & Hall Ltd. (2nd ed., pp. 1-140). Chapman & Hall.Quimio GM, Walter GH (2000) Swarming, delayed sexual maturation of males and mating behavior of Fopius arisanus (Sonan) (Hymenoptera: Braconidae). *Journal of Insect Behaviour*, 13: 797–813.

Ramadan MM, Wong TTY, Wong MA (1991) Influence of parasitoid size and age on male mating success of opiines (Hymenoptera: Braconidae), larval parasitoids of fruit flies. *Biological Control*, 1: 248–255.

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, 1312(1): 105-112.

Reitz, S. R., & Trumble, J. T. (2002). Competitive displacement among insects and arachnids. *Annual Review of Entomology*, 47(1): 435-465.

Rios-Velasco, C., Gallegos-Morales, G., Cambero-Campos, J., Cerna-Chávez, E., Del Rincón-Castro, M. C., & Valenzuela-García, R. (2011). Natural enemies of the Fall armyworm Spodoptera frugiperda (Lepidoptera: Noctuidae) in Coahuila, México. *The Florida Entomologist*, 94(3): 723-726.

Rivero, A., & Casas, J. (1999a). Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. *Researches on Population Ecology*, 41(1): 39-45.

Rivero, A., & Casas, J. (1999b). Rate of nutrient allocation to egg production in a parasitic wasp. Proceedings of the Royal Society of London. *Series B: Biological Sciences*, 266(1424): 1169-1174.

Rosenheim, J. A. (1996). An evolutionary argument for egg limitation. *Evolution*, 50(5): 2089-2094.

Ruiz-Najera, R.E.; Molina-Ochoa, J.; Carpenter, J.E.; Espinosa-Moreno, J.A.; Ruíz-Nájera, J.A.; Lezama-Gutiérrez, R.; Foster, J.E. (2007). Survey for Hymenopteran and Dipteran Parasitoids of the Fall Armyworm (Lepidoptera: Noctuidae) in Chiapas, Mexico.*Journal of Agricultural and Urban Entomology*, 24(1): 35–42.

Rodriguez, J. J., Fernández-Triana, J. L., Smith, M. A., Janzen, D. H., Hallwachs, W., Erwin, T. L., & Whitfield, J. B. (2013). Extrapolations from field studies and known faunas converge on dramatically increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). *Insect Conservation and Diversity*, 6(4): 530-536.

Roitberg BD, Boivin G, Vet LEM (2001) Fitness, parasitoids, and biological control: an opinion. *The Canadian Entomology*, 133: 429–438.

Sait, S. M., Begon, M., Thompson, D. J., Harvey, J. A., & Hails, R. S. (1997). Factors affecting host selection in an insect host–parasitoid interaction. *Ecological Entomology*, 22(2): 225-230.

Sanap, Y.; Dawkar, V.V.; Giri, A.P.; Sen, A.; Pandit, R.S. (2016). Parasitism by *Chelonus blackburni* (Hymenoptera) affects food consumption and development of *Helicoverpa armigera* (Lepidoptera) and cellular architecture of the midgut. *Journal of Asia-Pacific Entomol*ogy, 19: 65–70.

Schmidt, O.; Theopold, U.; Strand, M. (2001). Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. *BioEssays*, 23, 344–351.

Schnitger, A.K., Kafatos, F.C., Osta, M.A., (2007). The melanization reaction is not required for survival of *Anopheles gambiae* mosquitoes after bacterial infections. *Journal of Biological Chemistry*, 282: 21884–21888.

Sisay, B.; Simiyu, J.; Malusi, P.; Likhayo, P.; Mendesil, E.; Elibariki, N.; Wakgari, M.; Ayalew, G.; Tefera, T. (2018) First report of the Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), natural enemies from Africa. J. Appl. Entomol., 142(8), 800–804. DOI:10.1111/jen.12534.

Sisay, B., Tefera, T., Wakgari, M., Ayalew, G., & Mendesil, E. (2019). The efficacy of selected synthetic insecticides and botanicals against Fall armyworm, Spodoptera frugiperda, in maize. *Insects*, 10(2): 45. https://doi.org/10.3390/insects10020045

Sparks, A.N. (1979). A review of the biology of the Fall armyworm. *Florida Entomologist*, 62(2): 82–87.

Statistics Research and Information Directorate (SRID) (2018). Agriculture in Ghana: Facts and Figures (2018), 25th ed.; Report of MOFA (Ministry of Food and Agriculture); MOFA: Accra, Ghana, 113 pp.

Stearns, S. C. (1992). The evolution of life histories (Vol. 249, p. xii). Oxford: Oxford University Press.

Strand, M.R., Noda, T., (1991). Alterations in the haemocytes of *Pseudoplusia includens* after parasitism by *Microplitis demolitor*. *Journal of Insect Physiol*ogy, 37: 839–850.

Thomazoni, D.; Formentini, M.A.; Francisco, L.; Alves, A (2014). Pathogenicity of entomopathogenic fungi to *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *Arquivos do Instituto Biológico (São Paulo)*, 81: 126–133.

Van Noort, S. (2019). WaspWeb: Hymenoptera of the Afrotropical Region. Available online: *www.waspweb.org* (accessed on 5 MAY 2021).

Vickery, R. A. (1929). Studies on the Fall armyworm in the gulf coast district of Texas (No. 1488-2016-123295).

Wagner, S. C., Zablotowicz, R. M., Locke, M. A., Smeda, R. J., Bryson, C. T., Kingery, W. L., & Buehring, N. (1995). Influence of herbicide-desiccated cover crops on biological soil quality in the Mississippi Delta. *Conservation Farming: A Focus on Water Quality. MAFES Special Bulletin*, (88-7): 55-58.

Wang, X. G., Bokonon-Ganta, A. H., & Messing, R. H. (2008). Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: effect of co-evolutionary history on competitive superiority. *Biological Control*, 44(3): 312-320.

Wheeler, G. S., Ashley, T. R., & Andrews, K. L. (1989). Larval parasitoids and pathogens of the Fall armyworm in Honduran maize. *Entomophaga*, 34(3): 331-340.

Whitfield, J. B., Wharton, R. A., Marsh, P. M., & Sharkey, M. J. (1997). Subfamily microgastrinae. *Manual of the New World genera of the family Braconidae (Hymenoptera). Special Publication*, (1): 333-364.

Whitfield, J. B. (1998). Phylogeny and evolution of host-parasitoid interactions in Hymenoptera. *Annual Review of Entomology*, 43(1): 129-151.

Wilkes, A. (1966). Sperm utilization following multiple insemination in the wasp *Dahlbominus fuscipennis*. *Canadian Journal of Genetics Cytology,* 8(3): 451– 461.

Wiltshire, E. P. (1977). Middle-east Lepidoptera, XXXVII: Notes on the Spodoptera litura (F.)-Group (Noctuidae-Trifinae). *Proceedings and transactions. British Entomol*. *Natural History Society*. 10: 92–96.

Xu, H. Y., Yang, N. W., & Wan, F. H. (2013). Competitive interactions between parasitoids provide new insight into host suppression. *PloS One*, *8*(11): e82003.

Yamanaka, A.; Hayakawa, Y.; Noda, H.; Nakashima, N.; Watanabe, H. (1996) Characterization of polydnavirus-encoded mRNA in parasitized armyworm larvae. *Insect Biochemistry and Molecular Biology*, 26(6): 529–536.

Yu D. S. K, Van Achterberg C, Horstmann K (2016) Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. Nepean, Ontario, Canada. http://www.taxapad.com.

Yu, S. J. (1991). Insecticide Resistance in the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pesticide biochemistry and physiology* 39: 84-91.

APPENDICES

APPENDIX I

Generation time descriptive statistical analysis.

Coccygidium luteum

Descriptive Statistics: EGG TO LARVA, PUPA TO ADUL, ADULT LIFE S, EGG TO ADULT, EGG TO ADULT (days)

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
EGG TO LARVA	250	0	9.0240	0.0471	0.7441	7.0000	9.0000	10.0000
PUPA TO ADULT	250	0	9.6760	0.0437	0.6906	8.0000	10.0000	11.0000
ADULT LIFE SPAN	250	0	11.488	0.347	5.487	1.000	12.000	23.000
GENERATION TIME	250	0	30.044	0.386	6.104	0.000	30.000	42.000
EGG-ADULT EMERGENCE	250	0	18.700	0.0676	1.069	16.000	19.000	21.000

Descriptive Statistics: LARVA TO PUPA (hours)

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
LARVA TO PUPA	250	0	8.112	0.104	1.639	4.000	8.000	13.000

Two-Sample T-Test and CI: MALE LIFE SPAN, FEMALE LIFE SPAN

Two-sample T for MALE LIFE SPAN vs FEMALE LIFE SPAN

	N	Mean	StDev	SE Mean
MALE LIFE SPAN	150	10.89	5.17	0.42
FEMALE LIFE SPAN	150	10.73	4.64	0.38

Difference = μ (MALE LIFE SPAN) - μ (FEMALE LIFE SPAN)
Estimate for difference: 0.153
95% CI for difference: (-0.963, 1.270)
T-Test of difference = 0 (vs ≠): T-Value = 0.27 P-Value = 0.787 DF = 294

Cotesia icipe

Descriptive Statistics: EGG TO LARVA, PUPA TO ADUL, ADULT LIFE S, EGG TO ADULT, EGG TO ADULT (days)

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
EGG TO LARVA	250	0	6.8480	0.0647	1.0223	0.0000	7.0000	8.0000
PUPA TO ADULT	250	0	2.9720	0.0323	0.5102	0.0000	3.0000	4.0000
ADULT LIFE SPAN	250	0	7.168	0.258	4.075	0.000	7.000	15.000
EGG TO ADULT DEATH	250	0	16.988	0.293	4.625	0.000	17.000	25.000

```
EGG TO ADULT        250    0  9.8200   0.0904  1.4296   0.0000  10.0000  11.0000
```

Descriptive Statistics: LARVA TO PUPA (hours)

```
Variable       N   N*    Mean  SE Mean   StDev  Minimum  Median  Maximum
LARVA TO PUPA 25   0  3.7680   0.0921  1.4569   0.0000  4.0000 8.0000
```

Two-Sample T-Test and CI: MALE ADULT LIFE SPAN, FEMALE ADULT LIFE SPAN

```
Two-sample T for MALE ADULT LIFE SPAN vs FEMALE ADULT LIFE SPAN

                          N    Mean  StDev     SE Mean
MALE ADULT LIFE SPAN      150  7.14   3.50     0.29
FEMALE ADULT LIFE SPAN    150  8.34   3.99     0.33


Difference = μ (MALE ADULT LIFE SPAN) - μ (FEMALE ADULT LIFE SPAN)
Estimate for difference:  -1.200
95% CI for difference:  (-2.054, -0.346)
T-Test of difference = 0 (vs ≠): T-Value = -2.77  P-Value = 0.006  DF = 292
```

APPENDIX II

Preference test statistics.

Coccygidium luteum

No-choice test.

% PARASITISM

Descriptive Statistics: NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, ...

```
Variable  N  N*      Mean   SE Mean      StDev       Sum       Median   Maximum
NEW (0)   5   0     62.00      6.63      14.83    310.00        60.00     80.00
1 DAY     5   0     74.00      5.10      11.40    370.00        70.00     90.00
2 DAYS    5   0     82.00      3.74       8.37    410.00        80.00     90.00
3 DAYS    5   0     92.00      3.74       8.37    460.00        90.00    100.00
4 DAYS    5   0     98.00      2.00       4.47    490.00       100.00    100.00
5 DAYS    5   0     98.00      2.00       4.47    490.00       100.00    100.00
6 DAYS    5   0     86.00      2.45       5.48    430.00        90.00     90.00
7 DAYS    5   0     22.00      7.35      16.43    110.00        20.00     50.00
8 DAYS    5   0      2.00      2.00       4.47     10.0         0.00     10.00
9 DAYS    5   0  0.000000  0.000000  0.000000  0.000000     0.000000  0.000000
```

One-way ANOVA: NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	68272	7585.78	89.24	0.000
Error	40	3400	85.00		
Total	49	71672			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
9.21954	95.26%	94.19%	92.59%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
5 DAYS	5	98.00	A
4 DAYS	5	98.00	A
3 DAYS	5	92.00	A B
6 DAYS	5	86.00	A B
2 DAYS	5	82.00	A B
1 DAY	5	74.00	B C
NEW (0)	5	62.00	C
7 DAYS	5	22.00	D
8 DAYS	5	2.00	E
9 DAYS	5	0.000000	E

Significantly different means do not share the same letter.

% FAW MORTALITY

One-way ANOVA: NEW (0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, 7 DAYS_1, 8 DAYS_1, 9 DAYS_1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	8008	889.78	18.16	0.000
Error	40	1960	49.00		
Total	49	9968			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7	80.34%	75.91%	69.28%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
NEW (0)_1	5	38.00	A
1 DAY_1	5	26.00	A B
2 DAYS_1	5	16.00	B C
3 DAYS_1	5	8.00	C D
5 DAYS_1	5	2.00	C D
4 DAYS_1	5	2.00	C D
9 DAYS_1	5	0.000000	D
8 DAYS_1	5	0.000000	D
7 DAYS_1	5	0.000000	D
6 DAYS_1	5	0.000000	D

Significantly different means do not share the same letter.

% PARASITIOD ADULT EMERGENCE OUT OF PARASITIZED LARVA

One-way ANOVA: NEW (0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, 7 DAYS_2, 8 DAYS_2, 9 DAYS_2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	60742	6749.2	28.54	0.000
Error	40	9460	236.5		
Total	49	70202			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
15.3782	86.53%	83.49%	78.95%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
7 DAYS_2	5	100.0	A
6 DAYS_2	5	100.0	A
5 DAYS_2	5	100.0	A
3 DAYS_2	5	97.78	A
4 DAYS_2	5	96.00	A
2 DAYS_2	5	95.28	A
NEW (0)_2	5	90.48	A
1 DAY_2	5	87.14	A
8 DAYS_2	5	20.0	B
9 DAYS_2	5	0.000000	B

Significantly different means do not share the same letter.

Behavior test statistics

new (0)

One-way ANOVA: NA, AD, AND

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA, AD, AND


Analysis of Variance

Source   DF   Adj SS   Adj MS  F-Value  P-Value
Factor    2  33.3333  16.6667        *        *
Error   147   0.0000   0.0000
Total   149  33.3333


Model Summary

S     R-sq  R-sq(adj)  R-sq(pred)
0  100.00%    100.00%     100.00%


Means

Factor   N      Mean     StDev          95% CI
NA      50  0.000000  0.000000  (-0.000000, -0.000000)
AD      50  0.000000  0.000000  ( 0.000000,  0.000000)
AND     50     1.000     0.000  (    1.000,     1.000)

Pooled StDev = 0
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor   N      Mean  Grouping
AND     50     1.000  A
AD      50  0.000000    B
NA      50  0.000000      C

Significantly different means do not share the same letter.
```

1 day

One-way ANOVA: NA_1, AD_1, AND_1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA_1, AD_1, AND_1


Analysis of Variance

Source   DF   Adj SS   Adj MS  F-Value  P-Value
Factor    2  33.3333  16.6667        *        *
Error   147   0.0000   0.0000
Total   149  33.3333


Model Summary

S     R-sq  R-sq(adj)  R-sq(pred)
0  100.00%    100.00%     100.00%


Means

Factor   N      Mean     StDev          95% CI
NA_1    50  0.000000  0.000000  (-0.000000, -0.000000)
AD_1    50  0.000000  0.000000  ( 0.000000,  0.000000)
AND_1   50     1.000     0.000  (    1.000,     1.000)

Pooled StDev = 0
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor   N      Mean  Grouping
AND_1   50     1.000  A
AD_1    50  0.000000    B
NA_1    50  0.000000      C

Significantly different means do not share the same letter.
```

2 days

One-way ANOVA: NA_2, AD_2, AND_2

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA_2, AD_2, AND_2


Analysis of Variance

Source   DF   Adj SS   Adj MS  F-Value  P-Value
Factor    2  33.3333  16.6667        *        *
Error   147   0.0000   0.0000
Total   149  33.3333


Model Summary

S     R-sq  R-sq(adj)  R-sq(pred)
0  100.00%    100.00%     100.00%


Means

Factor   N      Mean     StDev          95% CI
NA_2    50  0.000000  0.000000  (-0.000000, -0.000000)
AD_2    50  0.000000  0.000000  ( 0.000000,  0.000000)
AND_2   50     1.000     0.000  (    1.000,     1.000)

Pooled StDev = 0
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor   N      Mean  Grouping
AND_2   50     1.000  A
AD_2    50  0.000000    B
NA_2    50  0.000000      C

Significantly different means do not share the same letter.
```

3 days

One-way ANOVA: NA_3, AD_3, AND_3

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA_3, AD_3, AND_3


Analysis of Variance

Source    DF   Adj SS   Adj MS  F-Value  P-Value
Factor     2  33.3333  16.6667        *        *
Error    147   0.0000   0.0000
Total    149  33.3333


Model Summary

S     R-sq  R-sq(adj)  R-sq(pred)
0  100.00%    100.00%     100.00%


Means

Factor   N      Mean     StDev          95% CI
NA_3    50  0.000000  0.000000  (-0.000000, -0.000000)
AD_3    50  0.000000  0.000000  ( 0.000000,  0.000000)
AND_3   50     1.000     0.000  (    1.000,     1.000)

Pooled StDev = 0
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor   N      Mean  Grouping
AND_3   50     1.000  A
AD_3    50  0.000000    B
NA_3    50  0.000000      C

Significantly different means do not share the same letter.
```

4 days

One-way ANOVA: NA_4, AD_4, AND_4

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA_4, AD_4, AND_4


Analysis of Variance

Source   DF  Adj SS   Adj MS  F-Value  P-Value
Factor    2   22.77  11.3867   158.51    0.000
Error   147   10.56   0.0718
Total   149   33.33


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.268024  68.32%     67.89%      67.01%


Means

Factor   N      Mean     StDev          95% CI
NA_4    50  0.000000  0.000000  (-0.074908, 0.074908)
AD_4    50    0.1200    0.3283  (   0.0451,   0.1949)
AND_4   50    0.8800    0.3283  (   0.8051,   0.9549)

Pooled StDev = 0.268024
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor   N      Mean  Grouping
AND_4   50    0.8800  A
AD_4    50    0.1200    B
NA_4    50  0.000000    B

Significantly different means do not share the same letter.
```

5 days

One-way ANOVA: NA_5, AD_5, AND_5

```
Method

Null hypothesis         All means are equal
```

```
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA_5, AD_5, AND_5


Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
Factor    2   12.33  6.1667    43.17    0.000
Error   147   21.00  0.1429
Total   149   33.33


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.377964  37.00%     36.14%      34.40%


Means

Factor   N      Mean     StDev          95% CI
NA_5    50  0.000000  0.000000  (-0.105634, 0.105634)
AD_5    50    0.7000    0.4629  (   0.5944,   0.8056)
AND_5   50    0.3000    0.4629  (   0.1944,   0.4056)

Pooled StDev = 0.377964
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor   N      Mean  Grouping
AD_5    50    0.7000  A
AND_5   50    0.3000    B
NA_5    50  0.000000      C

Means that do not share a letter are significantly different.
```

6 days

One-way ANOVA: NA_6, AD_6, AND_6

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05
```

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	NA_6, AD_6, AND_6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	27.613	13.8067	354.82	0.000
Error	147	5.720	0.0389		
Total	149	33.333			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.197260	82.84%	82.61%	82.13%

Means

Factor	N	Mean	StDev	95% CI
NA_6	50	0.0200	0.1414	(-0.0351, 0.0751)
AD_6	50	0.9400	0.2399	(0.8849, 0.9951)
AND_6	50	0.0400	0.1979	(-0.0151, 0.0951)

Pooled StDev = 0.197260

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
AD_6	50	0.9400	A
AND_6	50	0.0400	B
NA_6	50	0.0200	B

Significantly different means do not share the same letter.

7 days

One-way ANOVA: NA_7, AD_7, AND_7

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

```
Factor Information

Factor  Levels  Values
Factor       3  NA_7, AD_7, AND_7


Analysis of Variance

Source   DF  Adj SS   Adj MS  F-Value  P-Value
Factor    2   22.77  11.3867   158.51    0.000
Error   147   10.56   0.0718
Total   149   33.33


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.268024  68.32%     67.89%      67.01%


Means

Factor   N      Mean     StDev          95% CI
NA_7    50    0.1200    0.3283  (   0.0451,   0.1949)
AD_7    50    0.8800    0.3283  (   0.8051,   0.9549)
AND_7   50  0.000000  0.000000  (-0.074908, 0.074908)

Pooled StDev = 0.268024
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence
Factor   N      Mean  Grouping
AD_7    50    0.8800  A
NA_7    50    0.1200    B
AND_7   50  0.000000    B

Significantly different means do not share the same letter.
```

8 days

One-way ANOVA: NA_8, AD_8, AND_8

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  NA_8, AD_8, AND_8
```

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	9.773	4.8867	30.49	0.000
Error	147	23.560	0.1603		
Total	149	33.333			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.400340	29.32%	28.36%	26.41%

Means

Factor	N	Mean	StDev	95% CI
NA_8	50	0.3800	0.4903	(0.2681, 0.4919)
AD_8	50	0.6200	0.4903	(0.5081, 0.7319)
AND_8	50	0.000000	0.000000	(-0.111888, 0.111888)

Pooled StDev = 0.400340

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
AD_8	50	0.6200	A
NA_8	50	0.3800	B
AND_8	50	0.000000	C

Significantly different means do not share the same letter.

9 days

One-way ANOVA: NA_9, AD_9, AND_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	NA_9, AD_9, AND_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	18.57	9.2867	92.49	0.000
Error	147	14.76	0.1004		
Total	149	33.33			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.316872	55.72%	55.12%	53.89%

Means

Factor	N	Mean	StDev	95% CI
NA_9	50	0.8200	0.3881	(0.7314, 0.9086)
AD_9	50	0.1800	0.3881	(0.0914, 0.2686)
AND_9	50	0.000000	0.000000	(-0.088560, 0.088560)

Pooled StDev = 0.316872

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
NA_9	50	0.8200	A
AD_9	50	0.1800	B
AND_9	50	0.000000	C

Significantly different means do not share the same letter.

NA new to 9 days

One-way ANOVA: NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	32.60	3.62244	69.83	0.000
Error	490	25.42	0.05188		
Total	499	58.02			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.227766	56.19%	55.38%	54.38%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
NA_9	50	0.8200	A
NA_8	50	0.3800	B
NA_7	50	0.1200	C
NA_6	50	0.0200	C
NA_5	50	0.000000	C
NA_4	50	0.000000	C
NA_3	50	0.000000	C
NA_2	50	0.000000	C
NA_1	50	0.000000	C
NA	50	0.000000	C

Significantly different means do not share the same letter.

AD new to 9 days

One-way ANOVA: AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	69.79	7.75467	88.29	0.000
Error	490	43.04	0.08784		
Total	499	112.83			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.296373	61.85%	61.15%	60.28%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
AD_6	50	0.9400	A
AD_7	50	0.8800	A B
AD_5	50	0.7000	B C
AD_8	50	0.6200	C
AD_9	50	0.1800	D
AD_4	50	0.1200	D
AD_3	50	0.000000	D
AD_2	50	0.000000	D
AD_1	50	0.000000	D
AD	50	0.000000	D

Significantly different means do not share the same letter.

Tukey Simultaneous 95% CIs

AND new to 9 days

One-way ANOVA: AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	107.06	11.8953	329.31	0.000
Error	490	17.70	0.0361		
Total	499	124.76			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.190059	85.81%	85.55%	85.23%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AND_3	50	1.000	A
AND_2	50	1.000	A
AND_1	50	1.000	A
AND	50	1.000	A
AND_4	50	0.8800	A
AND_5	50	0.3000	B
AND_6	50	0.0400	C
AND_9	50	0.000000	C
AND_8	50	0.000000	C
AND_7	50	0.000000	C

Significantly different means do not share the same letter.

Choice test

One-way ANOVA: NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

% PARASITISM

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	43200	4800	2.67	0.016

```
Error    40   72000    1800
Total    49  115200


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
42.4264  37.50%     23.44%       2.34%
```

Fisher Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor    N      Mean  Grouping
5 DAYS    5      80.0  A
4 DAYS    5      80.0  A
3 DAYS    5      60.0  A B
6 DAYS    5      40.0  A B C
2 DAYS    5      40.0  A B C
1 DAY     5      40.0  A B C
NEW (0)   5      20.0    B C
9 DAYS    5  0.000000      C
8 DAYS    5  0.000000      C
7 DAYS    5  0.000000      C

Significantly different means do not share the same letter.
```

% FAW MORTALITY

One-way ANOVA: NEW (0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, ...

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor      10  NEW (0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6
DAYS_1, 7
                DAYS_1, 8 DAYS_1, 9 DAYS_1


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Factor   9   37000    4111     2.42    0.027
Error   40   68000    1700
Total   49  105000
```

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
41.2311	35.24%	20.67%	0.00%

Fisher Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
NEW (0)_1	5	80.0	A
2 DAYS_1	5	60.0	A B
1 DAY_1	5	60.0	A B
3 DAYS_1	5	40.0	A B C
6 DAYS_1	5	20.0	B C
5 DAYS_1	5	20.0	B C
4 DAYS_1	5	20.0	B C
9 DAYS_1	5	0.000000	C
8 DAYS_1	5	0.000000	C
7 DAYS_1	5	0.000000	C

difference new 0 and all, 2,1 and 7 to 9 days

Significantly different means do not share the same letter.

% ADULT PARASITOID EMERGENCE

One-way ANOVA: NEW (0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, 7 DAYS_2, 8 DAYS_2, 9 DAYS_2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	40800	4533	2.67	0.016

```
Error    40    68000    1700
Total    49   108800
```

```
Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
41.2311  37.50%     23.44%       2.34%
```

Fisher Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor      N      Mean  Grouping
5 DAYS_2    5      80.0  A
4 DAYS_2    5      80.0  A
6 DAYS_2    5      40.0  A B
2 DAYS_2    5      40.0  A B
1 DAY_2     5      40.0  A B
3 DAYS_2    5      20.0    B
NEW (0)_2   5      20.0    B
9 DAYS_2    5  0.000000    B
8 DAYS_2    5  0.000000    B
7 DAYS_2    5  0.000000    B

Significantly different means do not share the same letter.
```

Behavior statistics

NA NEW TO 9 DAYS

One-way ANOVA: NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor      10  NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Factor   9   76800  8533.3    10.67    0.000
```

```
Error    40    32000    800.0
Total    49   108800
```

```
Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
28.2843  70.59%     63.97%      54.04%
```

```
Means

Factor  N      Mean     StDev           95% CI
```

Fisher Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor  N      Mean  Grouping
NA_9    5     100.0  A
NA_8    5     100.0  A
NA_7    5      60.0     B
NA_6    5      40.0     B C
NA_5    5      20.0       C D
NA_4    5  0.000000         D
NA_3    5  0.000000         D
NA_2    5  0.000000         D
NA_1    5  0.000000         D
NA      5  0.000000         D

Significantly different means do not share the same letter.
```

AD NEW TO 9 DAYS

One-way ANOVA: AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor      10  AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
```

Factor	9	41800	4644.4	5.81	0.000
Error	40	32000	800.0		
Total	49	73800			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
28.2843	56.64%	46.88%	32.25%

Fisher Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
AD_5	5	80.0	A
AD_6	5	60.0	A B
AD_7	5	40.0	B
AD_9	5	0.000000	C
AD_8	5	0.000000	C
AD_4	5	0.000000	C
AD_3	5	0.000000	C
AD_2	5	0.000000	C
AD_1	5	0.000000	C
AD	5	0.000000	C

Means that do not share a letter are significantly different.

AND NEW TO 9DAYS

One-way ANOVA: AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	125000	13888.9	*	*
Error	40	0	0.0		
Total	49	125000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Fisher Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
AND_4	5	100.0	A
AND_3	5	100.0	B
AND_2	5	100.0	C
AND_1	5	100.0	D
AND	5	100.0	E
AND_9	5	0.000000	F
AND_8	5	0.000000	G
AND_7	5	0.000000	H
AND_6	5	0.000000	I
AND_5	5	0.000000	J

Means that do not share a letter are significantly different.

Cotecia icipe Preference

No-choice

% parasitism

One-way ANOVA: NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	74522	8280.2	73.28	0.000
Error	40	4520	113.0		
Total	49	79042			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
10.6301	94.28%	92.99%	91.06%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
1 DAY	5	94.00	A
2 DAYS	5	92.00	A
NEW (0)	5	76.00	A B
3 DAYS	5	58.0	B
4 DAYS	5	20.00	C
5 DAYS	5	6.00	C
9 DAYS	5	0.000000	C
8 DAYS	5	0.000000	C
7 DAYS	5	0.000000	C
6 DAYS	5	0.000000	C

Significantly different means do not share the same letter.

% ADULT PARASITOID EMERGENCE

One-way ANOVA: NEW (0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, 7 DAYS_1, 8 DAYS_1, 9 DAYS_1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	93520	10391.1	19.71	0.000
Error	40	21083	527.1		
Total	49	114604			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
22.9582	81.60%	77.46%	71.26%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
2 DAYS_1	5	100.0	A
3 DAYS_1	5	97.14	A
1 DAY_1	5	93.78	A
NEW (0)_1	5	82.70	A B
4 DAYS_1	5	80.0	A B
5 DAYS_1	5	40.0	B C
9 DAYS_1	5	0.000000	C
8 DAYS_1	5	0.000000	C
7 DAYS_1	5	0.000000	C
6 DAYS_1	5	0.000000	C

Significantly different means do not share the same letter.

% MORTALITY

One-way ANOVA: NEW (0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW (0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, 7 DAYS_2, 8 DAYS_2, 9 DAYS_2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	2088	232.00	5.27	0.000
Error	40	1760	44.00		
Total	49	3848			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6.63325	54.26%	43.97%	28.53%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
NEW (0)_2	5	24.00	A
1 DAY_2	5	6.00	B
7 DAYS_2	5	4.00	B
6 DAYS_2	5	4.00	B
5 DAYS_2	5	4.00	B
2 DAYS_2	5	4.00	B
9 DAYS_2	5	2.00	B
8 DAYS_2	5	2.00	B
4 DAYS_2	5	2.00	B
3 DAYS_2	5	0.000000	B

Significantly different means do not share the same letter.

No-choice Behavior

NA NEW TO 9 DAYS

One-way ANOVA: NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	68.59	7.6213	69.78	0.000
Error	490	53.52	0.1092		
Total	499	122.11			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.330491	56.17%	55.37%	54.36%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
NA_9	50	0.9600	A
NA_8	50	0.9600	A
NA_7	50	0.8400	A
NA_6	50	0.6000	B
NA_5	50	0.4200	B C
NA_4	50	0.2200	C D
NA_3	50	0.1800	D E
NA_2	50	0.0600	D E
NA_1	50	0.000000	E
NA	50	0.000000	E

Significantly different means do not share the same letter.

AD NEW TO 9 DAYS

One-way ANOVA: AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	30.57	3.3967	28.71	0.000
Error	490	57.98	0.1183		
Total	499	88.55			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.343986	34.52%	33.32%	31.82%

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
AD_4	50	0.7400	A
AD_5	50	0.5800	A B
AD_6	50	0.4000	B C
AD_3	50	0.2400	C D
AD_7	50	0.1600	D E
AD_2	50	0.1000	D E
AD_9	50	0.0400	D E
AD_8	50	0.0400	D E
AD_1	50	0.000000	E
AD	50	0.000000	E

Significantly different means do not share the same letter.

AND NEW TO 9 DAYS

One-way ANOVA: AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	92.32	10.2580	241.42	0.000
Error	490	20.82	0.0425		
Total	499	113.14			

Model Summary

```
       S    R-sq  R-sq(adj)  R-sq(pred)
0.206131  81.60%     81.26%       80.84%
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence
Factor   N      Mean  Grouping
AND_1   50     1.000  A
AND     50     1.000  A
AND_2   50    0.8400    B
AND_3   50    0.5800      C
AND_4   50    0.0400        D
AND_9   50  0.000000        D
AND_8   50  0.000000        D
AND_7   50  0.000000        D
AND_6   50  0.000000        D
AND_5   50  0.000000        D

Significantly different means do not share the same letter.
```

Cotesia icipe choice test

% PARASITISM

One-way ANOVA: NEW(0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS, 8 DAYS, 9 DAYS

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor      10  NEW(0), 1 DAY, 2 DAYS, 3 DAYS, 4 DAYS, 5 DAYS, 6 DAYS, 7 DAYS,
8 DAYS, 9 DAYS


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Factor   9   16200    1800     1.64    0.138
Error   40   44000    1100
Total   49   60200


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
33.1662  26.91%     10.47%       0.00%
```

Fisher Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
3 DAYS	5	60.0	A
4 DAYS	5	20.0	A B
2 DAYS	5	20.0	A B
1 DAY	5	20.0	A B
NEW(0)	5	20.0	A B
9 DAYS	5	0.000000	B
8 DAYS	5	0.000000	B
7 DAYS	5	0.000000	B
6 DAYS	5	0.000000	B
5 DAYS	5	0.000000	B

Significantly different means do not share the same letter.

% MORTALITY

One-way ANOVA: NEW(0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW(0)_1, 1 DAY_1, 2 DAYS_1, 3 DAYS_1, 4 DAYS_1, 5 DAYS_1, 6 DAYS_1, 7 DAYS_1, 8 DAYS_1, 9 DAYS_1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	56200	6244	4.46	0.000
Error	40	56000	1400		
Total	49	112200			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
37.4166	50.09%	38.86%	22.01%

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping
2 DAYS_1	5	80.0	A
1 DAY_1	5	80.0	A
NEW(0)_1	5	80.0	A
4 DAYS_1	5	40.0	A B
3 DAYS_1	5	40.0	A B
6 DAYS_1	5	20.0	B
9 DAYS_1	5	0.000000	B
8 DAYS_1	5	0.000000	B
7 DAYS_1	5	0.000000	B
5 DAYS_1	5	0.000000	B

Significantly different means do not share the same letter.

% ADULT PARASITOID EMERGENCE

One-way ANOVA: NEW(0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, ...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NEW(0)_2, 1 DAY_2, 2 DAYS_2, 3 DAYS_2, 4 DAYS_2, 5 DAYS_2, 6 DAYS_2, 7 DAYS_2, 8 DAYS_2, 9 DAYS_2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	8800	977.8	0.89	0.543
Error	40	44000	1100.0		
Total	49	52800			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
33.1662	16.67%	0.00%	0.00%

Fisher Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
4 DAYS_2	5	40.0	A
3 DAYS_2	5	20.0	A
2 DAYS_2	5	20.0	A
1 DAY_2	5	20.0	A
NEW(0)_2	5	20.0	A
9 DAYS_2	5	0.000000	A
8 DAYS_2	5	0.000000	A
7 DAYS_2	5	0.000000	A
6 DAYS_2	5	0.000000	A
5 DAYS_2	5	0.000000	A

Significantly different means do not share the same letter.

Behavior Statistics (choice)

NA NEW TO 9 DAYS

One-way ANOVA: NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	NA, NA_1, NA_2, NA_3, NA_4, NA_5, NA_6, NA_7, NA_8, NA_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	93000	10333.3	12.92	0.000
Error	40	32000	800.0		
Total	49	125000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
28.2843	74.40%	68.64%	60.00%

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping
NA_9	5	100.0	A
NA_8	5	100.0	A
NA_7	5	100.0	A
NA_6	5	80.0	A B
NA_5	5	60.0	B
NA_4	5	60.0	B
NA_3	5	0.000000	C
NA_2	5	0.000000	C
NA_1	5	0.000000	C
NA	5	0.000000	C

Significantly different means do not share the same letter.

AD NEW TO 9 DAYS

One-way ANOVA: AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	10	AD, AD_1, AD_2, AD_3, AD_4, AD_5, AD_6, AD_7, AD_8, AD_9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	13000	1444.4	1.81	0.097
Error	40	32000	800.0		
Total	49	45000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
28.2843	28.89%	12.89%	0.00%

Fisher Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping

```
AD_5     5      40.0  A
AD_4     5      40.0  A
AD_6     5      20.0  A B
AD_9     5  0.000000    B
AD_8     5  0.000000    B
AD_7     5  0.000000    B
AD_3     5  0.000000    B
AD_2     5  0.000000    B
AD_1     5  0.000000    B
AD       5  0.000000    B

Significantly different means do not share the same letter.
```

AND NEW TO 9 DAYS

One-way ANOVA: AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8, AND_9

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor      10  AND, AND_1, AND_2, AND_3, AND_4, AND_5, AND_6, AND_7, AND_8,
AND_9


Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value
Factor   9  120000  13333.3        *        *
Error   40       0      0.0
Total   49  120000


Model Summary

S     R-sq  R-sq(adj)  R-sq(pred)
0  100.00%    100.00%     100.00%
```

Fisher Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor  N      Mean  Grouping
AND_3   5     100.0  A
AND_2   5     100.0    B
```

```
AND_1    5     100.0      C
AND      5     100.0        D
AND_9    5  0.000000          E
AND_8    5  0.000000            F
AND_7    5  0.000000              G
AND_6    5  0.000000                H
AND_5    5  0.000000                  I
AND_4    5  0.000000                    J

Significantly different means do not share the same letter.
```

APPENDIX III

Parasitism Potential

PARASITISM POTENTIAL *Coccygidium luteum*

One-way ANOVA: PARSITISM, PARSITISM_1, PARSITISM_2, PARSITISM_3, PARSITISM_4

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       5  PARSITISM, PARSITISM_1, PARSITISM_2, PARSITISM_3, PARSITISM_4


Analysis of Variance

Source    DF  Adj SS   Adj MS  F-Value  P-Value
Factor     4   114.3  28.5800   194.30    0.000
Error   1195   175.8   0.1471
Total   1199   290.1


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.383530  39.41%     39.20%      38.89%


Means

Factor          N      Mean     StDev          95% CI
PARSITISM     250  0.000000  0.000000  (-0.047590, 0.047590)
PARSITISM_1   250    0.8200    0.3850  (   0.7724,   0.8676)
PARSITISM_2   250    0.7920    0.4067  (   0.7444,   0.8396)
PARSITISM_3   250    0.6600    0.4747  (   0.6124,   0.7076)
```

```
PARSITISM_4  200    0.7050    0.4572  (   0.6518,   0.7582)

Pooled StDev = 0.383530
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence
Factor          N      Mean  Grouping
PARSITISM_1   250    0.8200  A
PARSITISM_2   250    0.7920  A B
PARSITISM_4   200    0.7050    B C
PARSITISM_3   250    0.6600      C
PARSITISM     250  0.000000        D

Means that do not share a letter are significantly different.
```

One-way ANOVA: FAW MORTALIT, FAW MORTALIT, FAW MORTALIT, FAW MORTALIT, FAW MORTALIT

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       5  FAW MORTALITY, FAW MORTALITY_1, FAW MORTALITY_2, FAW
MORTALITY_3, FAW
                MORTALITY_4


Analysis of Variance

Source    DF   Adj SS   Adj MS  F-Value  P-Value
Factor     4   0.2967  0.07417     2.43    0.046
Error   1195  36.5000  0.03054
Total   1199  36.7967


Model Summary

       S   R-sq  R-sq(adj)  R-sq(pred)
0.174768  0.81%      0.47%       0.00%


Means

Factor             N     Mean    StDev          95% CI
FAW MORTALITY    250   0.0600   0.2380  (  0.0383,  0.0817)
FAW MORTALITY_1  250   0.0320   0.1764  (  0.0103,  0.0537)
FAW MORTALITY_2  250  0.02000  0.14028  (-0.00169, 0.04169)
```

```
FAW MORTALITY_3  250  0.01600  0.12573  (-0.00569, 0.03769)
FAW MORTALITY_4  200   0.0300   0.1710  (  0.0058,  0.0542)

Pooled StDev = 0.174768
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor              N     Mean  Grouping
FAW MORTALITY     250   0.0600  A
FAW MORTALITY_1   250   0.0320  A B
FAW MORTALITY_4   200   0.0300  A B
FAW MORTALITY_2   250  0.02000  A B
FAW MORTALITY_3   250  0.01600    B

Significantly different means do not share the same letter.
```

Cotesia icipe PARASITISM Potential

One-way ANOVA: PARASITISM, PARASITISM_1, PARASITISM_2, PARASITISM_3, PARASITISM_4

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       5  PARASITISM, PARASITISM_1, PARASITISM_2, PARASITISM_3,
PARASITISM_4


Analysis of Variance

Source    DF  Adj SS  Adj MS  F-Value  P-Value
Factor     4   36.45  9.1128    41.62    0.000
Error   1245  272.56  0.2189
Total   1249  309.02


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.467896  11.80%     11.51%      11.09%


Means

Factor          N    Mean   StDev       95% CI
PARASITISM    250  0.1880  0.3915  (0.1299, 0.2461)
```

```
PARASITISM_1  250  0.6080  0.4892  (0.5499, 0.6661)
PARASITISM_2  250  0.3360  0.4733  (0.2779, 0.3941)
PARASITISM_3  250  0.4560  0.4991  (0.3979, 0.5141)
PARASITISM_4  250  0.6480  0.4786  (0.5899, 0.7061)

Pooled StDev = 0.467896
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor          N    Mean  Grouping
PARASITISM_4  250  0.6480  A
PARASITISM_1  250  0.6080  A
PARASITISM_3  250  0.4560    B
PARASITISM_2  250  0.3360      C
PARASITISM    250  0.1880        D

Significantly different means do not share the same letter.
```

One-way ANOVA: FAW MORTALIT, FAW MORTALIT, FAW MORTALIT, FAW MORTALIT, FAW MORTALIT

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       5  FAW MORTALITY, FAW MORTALITY_1, FAW MORTALITY_2, FAW
MORTALITY_3, FAW
                MORTALITY_4


Analysis of Variance

Source    DF  Adj SS   Adj MS  F-Value  P-Value
Factor     4   7.141  1.78520    24.50    0.000
Error   1245  90.700  0.07285
Total   1249  97.841


Model Summary

       S   R-sq  R-sq(adj)  R-sq(pred)
0.269910  7.30%      7.00%       6.55%


Means

Factor             N    Mean   StDev       95% CI
```

```
FAW MORTALITY    250  0.2360  0.4255  (0.2025, 0.2695)
FAW MORTALITY_1  250  0.0440  0.2055  (0.0105, 0.0775)
FAW MORTALITY_2  250  0.0360  0.1867  (0.0025, 0.0695)
FAW MORTALITY_3  250  0.0560  0.2304  (0.0225, 0.0895)
FAW MORTALITY_4  250  0.0560  0.2304  (0.0225, 0.0895)

Pooled StDev = 0.269910
```

Tukey Pairwise Comparisons

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor             N    Mean  Grouping
FAW MORTALITY    250  0.2360  A
FAW MORTALITY_4  250  0.0560    B
FAW MORTALITY_3  250  0.0560    B
FAW MORTALITY_1  250  0.0440    B
FAW MORTALITY_2  250  0.0360    B

Significantly different means do not share the same letter.
```

Difference in *C. luteum* and *C. icipe* parasitism Potential.

Two-Sample T-Test and CI: C. luteum, C. icipe

```
Two-sample T for C. luteum vs C. icipe

           N   Mean  StDev  SE Mean
C. luteum  5   29.8   17.0      7.6
C. icipe   5  23.56   9.98      4.5


Difference = μ (C. luteum) - μ (C. icipe)
Estimate for difference:  6.21
95% CI for difference:  (-15.32, 27.74)
T-Test of difference = 0 (vs ≠): T-Value = 0.71  P-Value = 0.507  DF = 6
```

SEX RATIO

C. luteum

TOTAL MALE AND FEMALE IN 5 DAYS

Descriptive Statistics: MALE, FEMALE

```
Variable  N  N*   Mean  SE Mean  StDev     Sum  Minimum  Median  Maximum
MALE      5   0  97.40     6.67  14.91  487.00    86.00   87.00   117.00
FEMALE    5   0  42.20     3.50   7.82  211.00    33.00   42.00    54.00
```

DAILY COUNT *C. LUTEUM*

Descriptive Statistics: DAY 1 MALE, DAY 1- FEMALE, DAY 2, DAY2-, DAY 3, DAY3-, DAY 4, DAY4-, DAY 5, DAY5-

Variable	N	N*	Mean	SE Mean	StDev	Sum	Minimum	Median	Maximum
DAY 1 MALE	5	0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
DAY 1FEMALE	5	0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
DAY 2 MALE	5	0	24.80	2.37	5.31	124.00	20.00	23.00	33.00
DAY2 FEMALE	5	0	15.60	1.57	3.51	78.00	12.00	16.00	21.00
DAY 3 MALE	5	0	24.80	3.54	7.92	124.00	12.00	27.00	32.00
DAY3 FEMALE	5	0	14.40	2.18	4.88	72.00	11.00	13.00	23.00
DAY 4 MALE	5	0	26.00	3.51	7.84	130.00	12.00	29.00	30.00
DAY4 FEMALE	5	0	6.60	1.17	2.61	33.00	4.00	7.00	10.00
DAY 5 MALE	5	0	21.80	5.52	12.34	109.00	0.00	28.00	29.00
DAY5 FEMALE	5	0	5.60	1.78	3.97	28.00	0.00	7.00	9.00

COTESIA

TOTAL NUMBER OF MALE AND FEMALE IN 5 DAYS

Descriptive Statistics: MALLE, FEMALLE

Variable	N	N*	Mean	SE Mean	StDev	Sum	Minimum	Median	Maximum
MALLE	5	0	49.80	2.08	4.66	249.00	44.00	49.00	57.00
FEMALLE	5	0	62.00	4.09	9.14	310.00	48.00	63.00	70.00

DAILY COUNT

Descriptive Statistics: MALE_1, FEMALE_1, MALE_2, FEMALE_2, MALE_3, FEMALE_3, MALE_4, ...

Variable	N	N*	Mean	SE Mean	StDev	Sum	Minimum	Median	Maximum
MALE_1	5	0	8.80	2.52	5.63	44.00	0.00	10.00	14.00
FEMALE_1	5	0	0.600	0.400	0.894	3.000	0.000	0.000	2.000
MALE_2	5	0	11.60	1.25	2.79	58.00	9.00	11.00	15.00
FEMALE_2	5	0	18.80	1.46	3.27	94.00	14.00	20.00	22.00
MALE_3	5	0	8.000	0.316	0.707	40.000	7.000	8.000	9.000
FEMALE_3	5	0	8.80	1.24	2.77	44.00	6.00	8.00	13.00
MALE_4	5	0	9.800	0.735	1.643	49.000	7.000	10.000	11.000
FEMALE_4	5	0	13.000	0.837	1.871	65.000	11.000	13.000	16.000
MALE_5	5	0	11.60	1.36	3.05	58.00	8.00	11.00	16.00
FEMALE_5	5	0	20.80	2.35	5.26	104.00	14.00	20.00	27.00

APPENDIX IV

SEQUENTIAL INTRODUCTION (*C. luteum* FIRST)

Descriptive Statistics: C. icipe, C. luteum, FAW mortality

Variable	N	N*	Percent	Mean	SE Mean	StDev	Sum	Median
C. icipe	150	0	100	0.2933	0.0373	0.4568	44.0000	0.0000
C. luteum	150	0	100	0.2400	0.0350	0.4285	36.0000	0.0000
FAW mortality	150	0	100	0.4600	0.0408	0.5001	69.0000	0.0000

Two-Sample T-Test and CI: C. icipe, C. luteum

Two-sample T for C. icipe vs C. luteum

	N	Mean	StDev	SE Mean
C. icipe	150	0.293	0.457	0.037
C. luteum	150	0.240	0.429	0.035

Difference = μ (C. icipe) - μ (C. luteum)
Estimate for difference: 0.0533
95% CI for difference: (-0.0473, 0.1540)
T-Test of difference = 0 (vs ≠): T-Value = 1.04 P-Value = 0.298 DF = 296

SEQUENTIAL INTRODUCTION (*C. icipe* FIRST)

Descriptive Statistics: C. icipe_1, C. luteum_1, FAW mortality_1

Variable	N	N*	Percent	Mean	SE Mean	StDev	Sum	Median
C. icipe_1	150	0	100	0.2800	0.0368	0.4505	42.0000	0.0000
C. luteum_1	150	0	100	0.2333	0.0346	0.4244	35.0000	0.0000
FAW mortality_1	150	0	100	0.4533	0.0408	0.4995	68.0000	0.0000

Two-Sample T-Test and CI: C. icipe_1, C. luteum_1

Two-sample T for C. icipe_1 vs C. luteum_1

	N	Mean	StDev	SE Mean
C. icipe_1	150	0.280	0.451	0.037
C. luteum_1	150	0.233	0.424	0.035

Difference = μ (C. icipe_1) - μ (C. luteum_1)
Estimate for difference: 0.0467
95% CI for difference: (-0.0528, 0.1461)
T-Test of difference = 0 (vs ≠): T-Value = 0.92 P-Value = 0.357 DF = 296

CONCURRENT INTRODUCTION (*C. luteum* and *C. icipe* at the same time)

Descriptive Statistics: C. icipe_2, C. luteum_2, FAW mortality_2

Variable	N	N*	Percent	Mean	SE Mean	StDev	Sum	Median
C. icipe_2	150	0	100.000	0.1267	0.0272	0.3337	19.0000	0.0000
C. luteum_2	150	0	100.000	0.3067	0.0378	0.4627	46.0000	0.0000
FAW mortality_2	150	1	99.333	0.4400	0.0408	0.4984	66.0000	0.0000

Two-Sample T-Test and CI: C. icipe_2, C. luteum_2

Two-sample T for C. icipe_2 vs C. luteum_2

	N	Mean	StDev	SE Mean
C. icipe_2	150	0.127	0.334	0.027
C. luteum_2	150	0.307	0.463	0.038

Difference = μ (C. icipe_2) - μ (C. luteum_2)
Estimate for difference: -0.1800
95% CI for difference: (-0.2717, -0.0883)
T-Test of difference = 0 (vs ≠): T-Value = -3.86 P-Value = 0.000 DF = 271

PART TWO

DIFFERENCE IN COTESIA PARSITISM IN (COTE FIST , COTE SECOND, COTE IN CONCURENT AND COTE ONLY AS CONTROL)

One-way ANOVA: C. icipe_1, C. icipe, C. icipe_2, ONLY COTESIA

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	C. icipe_1, C. icipe 2ND, C. icipe BOTH, ONLY COTESIA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	54.59	18.1978	120.78	0.000
Error	596	89.80	0.1507		
Total	599	144.39			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.388164	37.81%	37.50%	36.97%

Means

Factor	N	Mean	StDev	95% CI
C. icipe_1	150	0.2800	0.4505	(0.2178, 0.3422)
C. icipe 2ND	150	0.2933	0.4568	(0.2311, 0.3556)
C. icipe BOTH	150	0.1267	0.3337	(0.0644, 0.1889)
ONLY COTESIA	150	0.9133	0.2823	(0.8511, 0.9756)

Pooled StDev = 0.388164

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
ONLY COTESIA	150	0.9133	A
C. icipe 2ND	150	0.2933	B
C. icipe_1	150	0.2800	B
C. icipe BOTH	150	0.1267	C

Significantly different means do not share the same letter.

DIFFERENCE IN *C. luteum* PARSITISM in (C. luteum FIST, C. luteum SECOND, C. luteum in CONCURENT AND C. luteum ONLY AS CONTROL)

One-way ANOVA: C. luteum, C. luteum_1, C. luteum_2, ONLY C. luteum

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	C. luteum 1ST, C. luteum 2ND, C. luteum BOTH, ONLY COCCY

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	34.11	11.3711	61.90	0.000
Error	596	109.48	0.1837		
Total	599	143.59			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.428592	23.76%	23.37%	22.73%

Means

Factor	N	Mean	StDev	95% CI
C. luteum 1ST	150	0.2400	0.4285	(0.1713, 0.3087)
C. luteum 2ND	150	0.2333	0.4244	(0.1646, 0.3021)
C. luteum BOTH	150	0.3067	0.4627	(0.2379, 0.3754)
ONLY COCCY	150	0.8067	0.3962	(0.7379, 0.8754)

Pooled StDev = 0.428592

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
ONLY COCCY	150	0.8067	A
C. luteum BOTH	150	0.3067	B
C. luteum 1ST	150	0.2400	B
C. luteum 2ND	150	0.2333	B

Significantly different means do not share the same letter.

Tukey Simultaneous 95% CIs

PART 3

TOTAL PARASITISM IN (COCCY FIRST, COTESIA FIRST, CONCURRENT, COTESIA ONLY AND COCCY ONLY)

One-way ANOVA: PARASITISM IN CO, PARASITISM IN CO, PARASITISM IN CO, ONLY COTESIA, ONLY COCCY

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	PARASITISM IN COTE FIRST, PARASITISM IN COCY FIRST, PARASITISM IN CONCURENT, ONLY COTESIA, ONLY COCCY

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	25.89	6.4733	32.83	0.000
Error	745	146.91	0.1972		
Total	749	172.80			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.444061	14.98%	14.53%	13.84%

Means

Factor	N	Mean	StDev	95% CI
PARASITISM IN COTE FIRST	150	0.5133	0.5015	(0.4422, 0.5845)
PARASITISM IN COCY FIRST	150	0.5333	0.5006	(0.4622, 0.6045)
PARASITISM IN CONCURENT	150	0.4333	0.4972	(0.3622, 0.5045)
ONLY COTESIA	150	0.9133	0.2823	(0.8422, 0.9845)
ONLY COCCY	150	0.8067	0.3962	(0.7355, 0.8778)

Pooled StDev = 0.444061

Tukey Pairwise Comparisons

Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor	N	Mean	Grouping
ONLY COTESIA	150	0.9133	A
ONLY COCCY	150	0.8067	A
PARASITISM IN COCY FIRST	150	0.5333	B
PARASITISM IN COTE FIRST	150	0.5133	B
PARASITISM IN CONCURENT	150	0.4333	B

Significantly different means do not share the same letter.

TOTAL FAW MORTALITY IN (COCCYGIDIUM FIRST, COTESIA FIRST, CONCURRENT, COTESIA ONLY AND COCCY ONLY)

One-way ANOVA: FAW mortality, FAW mortality, FAW mortality, COTESIA ONLY mortality, COCCYGIDIUM ONLY MORTALITY,...

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05
Rows unused	1

Equal variances were assumed for the analysis.

Factor Information

```
Factor  Levels  Values
Factor       6  FAW mortality COCCY FIRST, FAW mortality COTESIA FIRST, FAW
mortality
                CONCURRENTLY, COTESIA ONLY MORTALITY, COCCY ONLY MORTALITY,
CONTROL  FAW
                MORTALITY


Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
Factor    5   33.16  6.6313    41.61    0.000
Error   893  142.31  0.1594
Total   898  175.46


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.399194  18.90%     18.44%      17.80%


Means

Factor                          N    Mean   StDev         95% CI
FAW mortality COCCY FIRST     150  0.4600  0.5001  ( 0.3960, 0.5240)
FAW mortality COTESIA FIRST   150  0.4533  0.4995  ( 0.3894, 0.5173)
FAW mortality CONCURRENTLY    149  0.4430  0.4984  ( 0.3788, 0.5071)
COTESIA ONLY MORTALITY        150  0.0467  0.2116  (-0.0173, 0.1106)
COCCY ONLY MORTALITY          150  0.1733  0.3798  ( 0.1094, 0.2373)
CONTROL  FAW MORTALITY        150  0.0200  0.1405  (-0.0440, 0.0840)

Pooled StDev = 0.399194
```

Tukey Pair-wise Comparisons,,

```
Grouping results Using the Tukey mean separation Method and 95% Confidence

Factor                          N    Mean  Grouping
FAW mortality COCCY FIRST     150  0.4600  A
FAW mortality COTESIA FIRST   150  0.4533  A
FAW mortality CONCURRENTLY    149  0.4430  A
COCCY ONLY MORTALITY          150  0.1733    B
COTESIA ONLY MORTALITY        150  0.0467    B C
CONTROL  FAW MORTALITY        150  0.0200      C

Significantly different means do not share the same letter.
```