Larvicidal efficacy of Coconut Fruit Fiber (*Cocos nucifera* L.*)* and Pineapple peels (*Ananas comosus* L.) Extracts on 3rd instar larvae of *Culex quinquefasciatus* Mosquitos.

By

Osime Jeffery ESESE

(LSC1704890)

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Supervisor

Dr. Omoregie, A O

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CERTIFICATION

This is to certify that this project research was carried out by **Osime Jeffery ESESE** in the Department of Animal and Environmental Biology, Faculty of Life Sciences.

_____ _____

Dr. Omoregie, A O **Date**

(Project Supervisor)

_____ _____

Prof. (Mrs.) A.A Imaseun **Date**

(Date Head of Department)

DEDICATION

I dedicate my project research to my Father God for His Divine strength, favor, mercy, grace and wisdom upon me. I also dedicate it to my parents for their moral and financial support.

ACKNOWLEGEMENT

I express my profound gratitude to my Father God for his enabling grace and strength. I also appreciate all the various persons that have in one way or the other contributed r to the successful completion of my project work. My gratitude goes to my project supervisor Dr. Omoregie, A O for his constructive supervision which helped to bring out the best in me and opened me up to other aspects of this project, Thank you very much Sir. I deeply appreciate your candid support. Also my sincere gratitude goes to the Head of Department Prof. (Mrs.) A.A Imaseun for her academic and moral support. My gratitude also goes to all my lecturers Prof. Mike Omogberale, Dr. Nosakhare O. Erhunmwuse and Dr. I.N. Egbon. I wish to express my gratitude to my faithful parents Mr. and Mrs. ESESE for their moral and financial support. I also appreciate my siblings whose love and support has brought me this far and my wonderful friends in the department of Animal and Environmental Biology Major, Mary, Amarachi, precious, Ehis and izegbe who supported me in every way they could. I am truly grateful and privileged to have you all. May God bless and reward you all for your support and patience.

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ABSTRACT

The over use of synthetic insecticides for mosquito control, contaminates natural ecosystem and its inhabitants. Botanical resources are promising alternatives for pests and vector control due to their environmental compatibility, high biodegradability, target specificity, and zero resistance culture. Therefore this study investigated the larvicidal potency of aqueous and ethanolic extracts of *Cocos nucifera* fiber (CNF), *Ananas comosus* peels (ACP) and their synergy of 1:1 mixture against *Cx. quinquefasciatus* larvae. Qualitative phytochemistry of both plants were examined. *Cx. quinquefasciatus* 3rd instar larvae were subjected to different concentrations of 500ppm, 750ppm and 1000ppm for 24, 48 and 72hrs exposure time. Data was analysed statistically using Analysis of Variance (ANOVA). Phytochemical constituents revealed alkaloids, tannins, flavonoids and steroids present in equal strength in ethanolic CNF and ACP, Presence of flavonoids and Glycosides were both common in aqueous CNF and ACP. At 1000ppm (ECNF) extracts and (EACP) showed percentage mortality of 46.67% and 33.33% after 72hrs. Synergistic ethanoic extracts showed 53.33% mortality and 30% for the aqueous extracts under same conditions. The ethanolic combination of CNF and ACP recorded the lowest LC_{50}; 983.99ppm and LC_{90}; 1703.40ppm. While ethanolic ACP had the highest LC_{50}; 8676.96ppm, aqueous ACP had highest LC_{90}; 272866.3ppm respectively. There was significant mortality $P<0.05$ at 48 and 72hrs in (ECNF) and (EACP). The ethanolic synergistic extracts showed highest significant mortality $P<0.05$ at 48hrs of exposure. Results of synergistic CNF and ACP showed fair mortality on *Culex quinquefasciatus* larvae.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Globally, mosquitoes (Insecta: Diptera) are a wide spread nuisance species. They are opportunistic blood feeding insects that feed on both humans and livestock. This increases their potential to transmit zoonotic diseases (Rajkumar *et al.,* 2011). Generally mosquitos consist mainly four genera; *Culex, Mansonia, Anopheles*, and *Aedes* species. They are known to transmit a variety of disease of public health importance such as Malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE) amongst several others (Peace *et al.,* 2012; Ashfaq and Ashfaq, 2012; Jones *et al.,* 2012) resulting in millions of illness and deaths annually, in both humans and animals (Rahuman *et al.,* 2008). Chances of humans contacting Vector-borne diseases are on the increase due to the rapid urbanization in many developing countries and global climatic change. Possible breeding sites of mosquito vectors are now stationed around human dwellings (Intirach *et al.,* 2012; Karunamoorthi *et al.,* 2010).

Culex mosquitoes are pan tropical and subtropical disease vector and abundant in prevailing urban and rural areas. One of the most important groups of the *Culex* species is *Culex* pipiens complex, which comprise members namely; *Cx. quinquefasciatus, Cx. pallens, Cx. australicus, Cx. pipiens, Cx. globocoxitus and Cx. molestus* (Zittra *et al.,* 2019*). Culex quinquefasciatus* (the house misquote) is the most dominant spp in sub-Saharan Africa where thy habit wet pits, puddle, standing open drainage and other water bodies choked with organic debris to lay their eggs and larval stage development (Abdulaziz *et al.,* 2018; Jones *et al.,* 2012). They are the most important vector of the parasite *Wuchereria bancrofti* that causes Lymphatic filariasis, Louis elephantiasis virus. According to WHO, in 2022 over 860 million people in 47 countries worldwide remain

threatened by lymphatic filariasis. In Africa the prevalence of lymphatic filariasis (LF) is of rising concern. Elephantiasis of the limbs and urogenital disorders such as hydrocele in men has reportedly reached clinical status in 82 countries of the world, of which 34 countries are in Africa (Adekunle *et al.,* 2016). Several other millions of people suffering in sub-Saharan regions (Yadouléton *et al.,* 2015). According to the federal ministry of health, in large parts of Nigeria, LF is endemic. Statically, results from the past decades rates Nigeria as the third most endemic country after India and Indonesia, with estimate of 80-120 million at risk of infection (Lindsay and Thomas, 2000; Davies *et al.,* 2021).

Vector control has been and still is a major concern of public health importance worldwide. Conventional approaches towards vector control such as biological control and cultural control methods has been implemented over the past decades. The most effective methods were the introduction of synthetic chemical insecticides of the organophosphate and pyrethroid groups (Omotayo *et al.,* 2021). The lymphatic filariasis (LF) program that prevailed in 1994 along with the mass drug administration scheme to treat infected people in 2001 was associated with the lunch of the global program to eliminate lymphatic filariasis (LF) in many African countries deploying integrated vector management program (IVMP) (Mohammed *et al.,* 2006). This strategy involved the use of residual indoor insecticides sprays (IRS) and insecticides treated nets (ITN) as major tools (Sougoufara *et al.,* 2017). The success of the program endorsed chemical approach as a proficient vector control method, as significant decline of mosquito borne diseases especially malaria diseases were recorded (Yadouleton *et al.,* 2015; Chioma *et al.,* 2022). This strategy was quickly and widely adopted as a conventional method of vector and dominated over other control methods such as the non-chemical and biological control (Nawabor *et al.,* 2017). However, planning and effective implementation of chemical control measures requires a sound knowledge

of vector distribution, biology, changing trends of vector, as well as compounds or previous methods used and available. Although in many African countries, these tools may have had indirect effects on the changing trends or selection pressure on mosquito larvae (Chioma *et al.,* 2022).

Due to the wide spread use of synthetic insecticides over the last 20 years, the pyrethroids and organophosphate group has been intensely utilized by the public and farmers to control agricultural pests. Repetitive and indiscriminate use of synthetic insecticides for mosquito control disrupts natural ecosystems and the biological control systems, this led to reemergence of high mosquito populations (Das *et al.,* 2007). This practice has contaminated many breeding sites of mosquitoes, and has brought about the death of several other non-target species and resistance in many mosquitoes species. According to (Yadouléton *et al.,* 2015) the DDT indoor residual spray for malaria control was suspected of favoring the selection of DDT resistance in Anopheles and *Culex quinquefasciatus.* The resistance of *Culex* spp to different insecticides has been reported in different parts of Africa and other parts of the world (Yadouleton *et al.,* 2015; Agbor *et al.,* 2020; Scott *et al.,* 2015). The continual reliance on insecticides for vector control pose great risk to human health and social economic status of the globe. These limitations have resulted in the continued search for environmentally safe, effective, biodegradable and target-specific means of controlling vector population with no developmental resistance (Abdulaziz *et al.,* 2018; Shivakuma *et al.,* 2013).jn

Scientist have begun to appreciate the rich botanical resources as an alternative to synthetic insecticides to control vectors and insect pests largely. An estimate of about 2000 species of terrestrial plants as been tested for their insecticidal properties against vectors (Nanyonga *et al.,* 2012; Cheng *et al.,* 3013). Plants contain a wide range of potential phytochemicals such as

flavonoids, saponies, steroids, tannins, isoflavonoids, terpenes, etc. that can inflict mortality on insects at any stage of development: adults, ova, and larvae), they work by a variety of approach affecting one or more biological systems, including neurological, respiratory, and endocrine systems, as well as water balance of targeted species. As oppose to synthetic pesticides, they are rapidly biodegradable, ecofriendly, less toxic to human health, more target specific and shows zero resistance (Isman, 2006; Zhu *et al.,* 2008; Omena *et al.,* 2007; Ghosh *et al.,* 2012 WHO 2004, 2015). Various parts of plants such as roots, stem, bark, leaves, fruit, fruit seed and peels are used as botanicals. The success of Botanicals against mosquito vectors has brought attention and progressive research to the use of plant based chemicals for vector control.

Coconut (*Cocos nucifera*) commonly called coconut palm tree is a flourishing plant and an important member of the family Aracaceae and only accepted species of the genus Cocos. A matured coconut tree is around 25m in height, and has dense canopy. Coconut is an arborescent monocotyledonous plant, fruit comprises an outer outer skin called epicarp, a mesocarp which appears heavy, fibrous, and tanned when dry which can be used industrially, and an inner endocarp. Coconut tree is majorly grown in Africa and other parts of the world as a food source, decoration as well as for its culinary and non-culinary uses. The various parts of *Cocos nucifera* has several purposes including food, drinks, fibres, building materials. *Cocos nucifera* it has also been used as traditional medicine to treat arthritis and diarrhea, and for biological activities such as antimicrobial, antiviral, antinociceptive, anti-inflammatory, antineoplasic antioxidant and antioxidant (Shettigar *et al.,* 2014; Floriana *et al.,* 2015; Dabesor *et al.,* 2017).

Pineapple *(Ananas comosus)* belonging to the Bromeliaceae family, so named after the enzyme bromelain, which is one of its most important health promoting compounds (Salve and Ray, 2020). It is the third most important tropical fruit produced globally after bananas and mangos. It is native

to South America in Brazil and Paraguay, and also cultivated in some parts of Africa with Nigeria being one of the major Pineapple producing countries (Hikal *et al.,* 2021). It is grown largely for food. The fibrous flesh of *A. comosus* has a vibrant tropical flavour that balances the testes of sweet and tart (Piana *et al.,* 2005). Pineapple fruit are also consumed fresh, cooked, juiced, or preserved. Pineapple is very rich in essential nutrients such as potassium, calcium, phosphorus and iron, vitamin C, copper, folate, glycans and fibers. They also contain phytochemicals that exhibit antibacterial, antiviral, antifungal, ant parasitic and anti-inflammatory properties due to presence of polyphenols, flavonoids, saponins and other secondary metabolites present in the extract. Flavonoids and polyphenols are more potent in inhibiting gram-positive bacteria (Dabesor *et al.,* 2017; Hikal *et al.,* 2021).

1.2 Justification of study

Due to the limitations and negative effect of synthetic insecticides, the use of bio pesticides present in plant based materials as an alternative vector control is gaining grounds as a widely accepted and ecofriendly means of vector control. It has also been reported that over 2000 plant species has been tested for larvicidal effect against misquotes vectors (Cheng *et al.,* 3013). *Cocos nucifera,* and *Ananas comosus* are amongst the most common plants in Africa, Nigeria. Previous study has reported the insecticidal properties of *Cocos nucifera* coil and shell on *An. Stephensi, Aedes aegypti* and *Cx. quinquefasciatus (*Mohana *et al.,* 2012). The fiber of *Cocos nucifera* has been reported for anti-bacterial properties (Davi *et al.,* 2013). Also, *Ananas comosus* peels, has been reported to have repellence peoperties against *Aedes aegypti* (Rafiki and Nindia, 2022). The female cattle ticks *Rhipicephalus microplus showed* oviposition inhibition, when exposed to *Ananas comosus* peels (Luciana *et al.,* 2013). The fibers of *Cocos nucifera* and peels of *Ananas comosus*

are not widely utilized by public, but regarded as waste materials. Other studies have evaluated the anti-bacterial and activities of Cocos *nucifera,* and *Ananas comosus* owning the presence of varying phytochemicals in them (Hikal *et al.*, 2021; Dabestor *et al.*, 2017). Although no reported work has testing the efficacy of *Cocos nucifera* fiber, and *Ananas comosus* peels against the filarial vector *Cx. quienquefasciatus*, hence this study to access the insecticidal potentials of *Cocos nucifera* fibers and peels of *Ananas comosus,* and their synergistic effect against *Cx. quienquefasciatus.*

1.3 Aim and objective of Study

The aim of this study is to evaluate the larvicidal efficacy of two traditional plants extracts; Coconut (*Cocos nucifera* L.*)* Fiber and Pineapple (*Ananas comosus* L.) peels on 3rd instar larvae of *Culex* mosquito spp.

Specific objective of study:

1. To determine the phytochemical properties of *Cocos nucifera* fiber and *Ananas comosus peels*.
2. To determine the larvicidal activity of aqueous and ethanol extract of *Cocos nucifera* L. Fiber on 3rd instar *Culex* mosquito larvae.
3. To determine the larvicidal activity of aqueous and ethanol extract of *Ananas comosus* L. on 3rd instar *Culex* mosquito larvae.
4. To evaluate the effect of synergistic aqueous *Cocos nucifera* L. Fiber and *Ananas comosus* L. extract on 3rd instar *Culex* mosquito larvae.

5. To evaluate the effect of synergistic ethanol *Cocos nucifera* L. Fiber and *Ananas comosus* L. extract on 3rd instar of *Culex* mosquito larvae.
6. To determine the LC_{50} and LC_{90} value of Cocos *nucifera* L. Fiber extracts against 3rd instar of *Culex* mosquito larvae.
7. To determine the LC_{50} and LC_{90} value of *Ananas comosus* L. extracts on 3rd instar of *Culex* mosquito larvae.
8. To determine the LC_{50} and LC_{90} value of synergistic *Cocos nucifera* L. Fiber and *Ananas comosus L*. peels extracts on 3rd instar of *Culex* mosquito larvae.

CHAPTER TWO

2.0 LITERATURE REVIEW

Fruit Peels and Bark as Effective Botanicals.

The fruit peels of various fruits has been tested on different mosquito species, as well as other vectors and reported to have larvicidal, pupicidal and repellant properties against larval, pupal and adult stages respectively when exposed to certain test concentrations of extracts.

(Lima *et al.,* 2020) tested crude and methanolic extract of milled peels of unripe Avocado fruit peels (*Persea Americana Mill*) against the 3rd instar larvae of *Culex quinquefasciatus, Anopheles stephensi and Ades aegypti* for 24hrs. Concentrations used were 25, 50, 100, 150 and 250 ppm. The motality after 24hrs of exposure gave LC_{50} and LC_{90} values of 6.65 and 71.62 ppm for *Anopheles stephensi, Aedes aegypti* with 7.12 and 86.59 ppm and *Culex quinquefasciatus* with 10.78 and 69.39 ppm respectively. This study revealed mathanolic extract of unripe *Persea Americana* fruit peels is more potent against *Anopheles stephensi* as larvicide followed by *Aedes aegypti*, and showed the lest motality against *Culex quinquefasciatus* 3rd instar stage. There was a significant $P < 0.05$ in lethal concentrations.

Another study by (Shoymol and Joy, 2021) tested ethyl acetate, petroleum ether, and distilled water extracts of the ripe and unripe *Musca paradisiaca* against 4th instar larvae of malaria vector *Anopheles stephensi*. Exposure period of 24hrs with concentration (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 and 0.3125 mg/ml). Mortality after 24hrs; LC_{50} of ripe *Musa paradisiaca* peel extracts against *Anopheles stephensi* were 3.21, 2.55 mg/ml, while the unripe fruit peel extracts were 59.82, 48.08 mg/ml. LC_{90} of ripe *Musa paradisiaca* peel extracts against *Anopheles stephensi* were 4.8, 4.19 mg/ml, while unripe fruit peel extracts were 161.1, 122.22 mg/ml, respectively.

This demonstrated the fruit peels of *Musa paradisiaca* show promising larvicidal activity against malaria vectors.

(Mallick *et al.,* 2016) also reported fruit peels of *Citrus maxima* against 3rd instar *Cx. quinquefasciatus* mosquitos. n- hexane, ethyl acetate, and methanol solvent extracts were tested. Test solution of different concentrations of n-hexane, ethyl acetate and methanol fruit peel extract of 200, 400, 600, 800 and 1000ppm, were used. Percentage Larval mortality were recorded after 24, 42 and 72h respectively. 100% mortality of 3rd instar larvae were observed at 400 ppm concentration of n-hexane fruit peel extract after 24hrs of exposure however, ethyl acetate and methanol fruit peel extracts showed 100% mortality at 800 ppm concentration after 24 and 72hrs of exposure. LC_{50} values of n-hexane, ethyl acetate and methanol fruit peel extracts were 204.60, 640.95, and 336.36 ppm, respectively after 24 h of exposure. Fruit peels of *Citrus maxima* can serve as good botanical control against *Cx. quinquefasciatus.*

In 2011, Rajakumar *et al,* used the aqueous peels of *Annona squamosa* as larvicidal against *Anopheles subpictus* and *Cx. quinquefasciatus,* and futhered demonstrated *Annona squamosa* peels as Acaricidal, against adults of *Haemaphysalis bispinosa* (Acarina: Ixodidae). The observed larvicidal efficacies were 36, 55, 72, 92, 100% and 14, 34, 68, 89, and 100% at concentrations of 200, 400, 600, 800, and 1,000 ppm, respectively. The (LC_{50}=327.27ppm, r2= 0.970) in *An. subpictus* and (LC_{50}=456.29 ppm, r2= 0.974) in *Cx. quinquefasciatus*, the $\chi 2$ values were reported to be significant $p<0.05$ level. This study was concluded giving that the fruit peels of *A. squamosa*, may be an alternative to synthetic pesticides, particularly in dealing with blood and medically important vector such as mosquitos.

(Kadarkarai *et al.*, 2012) demonstrated the pupicidial, repellent and aduticidial properties of *Citrus sinensis Anopheles stephensi, Aedes aegypti and Cx. quinquefasciatus* after 24hrs of exposure to

concentration ranging from 100, 200, 300, 400 and 500 ppm, respectively. The resulting (LC_{50}) for the larvicidal and pupicidal activities of ethanoic extract of *C. sinensis* against *An. stephensi* first to fourth instar larvae and pupae were 182.24, 227.93, 291.69, 398.00 and 490.84 ppm; *Aedes aegypti* values were 92.27, 106.60, 204.87, 264.26, 342.45, 436.93 and 497.41 ppm; and *Cx. quinquefasciatus* were 244.70, 324.04, 385.32, 452.78 and 530.97 ppm, respectively. Adult mortality was recorded in ethanolic *C. sinensis* extract with LC_{50} and LC_{90} values of 272.19 and 457.14 ppm, *An. Stephensi*; 289.62 and 494.88 ppm, *Aedes aegypti*; and 320.38 and 524.57 ppm, respectively.

Cocos nucifera fiber Botanicals

C. nucifera fiber are used industrially for production of carpet, as fertilizers for agriculture, and sculpture for art. In Brazil, extracts of *C. nucifera* are used to treat diarrhea, leaves of *C. nucifera* are also chewed to treat diarrhea and stomach upset. In places in Papua New Guinea. *C. nucifera* husk fiber extract is used as antipyretic, to reduce renal inflammation, and as a topic ointment for dermatitis, abscesses, and injuries in Guatemala (Caceres *et al.,* 1987; Winger *et al.,* 1986). Phytochemical screen of the ethanolic coconut fiber (mesocarp) extract revealed the presence of phenols, tannins, leucoanthocyanidins, flavonoids, triterpenes, steroids, and alkaloids (Dabestor *et al.,* 2017), which has been used as oxidants and botanicals for vector control.

(Davi *et al.,* 2013) carried out a study was to investigate the antimicrobial activity of aqueous extracts and fractions from the husk fiber of the *C. nucifera* against bacteria (Staphylococcus aureus, S. aureus MRSA) and fungi (Candida albicans, Cryptococcus neoformans, Trichophyton rubrum and Fonsecaea pedrosoi). Result showing *C. nucifera* possess Antibacterial properties.

Modern medical science is now confirming the medicinal qualities which are used for the treatment of heart, liver and kidney disorders. Due to its contents of caprylic acid, which is fungicidal, is used in the treatment of fungal skin infections such as athlete's foot, thrush, ringworm and candidiasis (Fife, 2000). *C. nucifera* produced different products which include coconut water, coconut husk, copra, coconut oil, raw kernel, coconut cake and coconut milk. It is a unique source of different natural products in the development of drugs and industrial products that is effective against fungi, bacteria, viruses, parasites and dermatophytes (Floriana *et al.*, 2015).

A study by Mohana *et al.*, (2012) using silver nanoparticles from *Cocos nucifera* (coconut) coir extract against larvae of *An. stephensi* and *Cx. quinquefasciatus*. The various concentration used were ranging from 4,2,1,0.5 and 0.25mg/l for synthesized silver nanoparticles (AgNPs), targeted mosqutoes species was exposed to concentration for 72 hrs. The LC_{50} and LC_{90} values of *An. stephensi* were (LC50 =87.24 ± 4.75 mg/L; LC90 = 230.90 ± 17.10 mg/L), for *Cx. quinquefasciatus* (LC50 = 49.89 ± 2.42 mg/L; LC90 = 84.85 ± 6.50 mg/L).The acute toxicity was recorded at the end of 24hrs. The result confirmed significant $p<0.05$ susceptibility to synthesized silver nanoparticles at 72hrs of exposure

Gomathi *et al.,* (2019) demonstrated another study buy assessing the silver nanoparticles from *Cocos nucifera* (coconut) Shell against Dengue Vector (*Aedes aegypti*). The various test concentrations used were 2, 4, 6, 8 and 10 ppm of silver nanoparticle. Mortality was recorded after every 24hrs for a period of 12 days. The end result revealed the highest mortality rate in concentration of 10ppm against mosquitos larval. High mortality was inflected, and the detoxifying enzymatic profiles (Glutathione S transferase and Cytochrome P450). The result confirmed that *Cocos nucifera* (coconut) Shell is an ecofriendly, and nontoxic source for synthesis of silver nitrate particles and are highly efficient against *Ae. aegypti*.

A study by Nazaire *et al.* (2021) in Dogbo district south-western Benin, West Africa tested larvicidal effect of coconut oil. *A gambiae* were exposed to test concentrations for a period of 72hrs. After 24hrs, test blows recorded no alive larvae but 6, 9 and 5 moribund larval. After 48hrs, moribund larval reduced to 3, 0 and 1, then after 72hrs, there was no moribund larval, as all were said to have died. This study concluded that the presence of coconut oil aftects siphonal respiration of mosquito larvae and may be an effective larvicide.

Ananas comosus peel Botanicals

The extract of *Ananas comosus* peels has revealed the presence of phytochemicals such as alkaloid, flavonoid, tannin, cardiac glycoside, saponin and phlobatannin in them (Manimozhi *et al.,* 2012). Which makes them very important. Bromelain, a protease found in pineapples, is of high demand in the pharmaceutical, cosmetic and in the food industries, which use as a meat tenderiser, anti-browning agent and in the production of infant formulas (Tochi *et al.,* 2008). The biosynthesis of metal nanoparticles has been reported by (Ahmad and Sharm, 2012) using aqueous *Ananas comosus* extract and had successfully produced silver and cooper nanoparticles. Which were used against mosquito vectors. They are also used in clinical department for wound healing and as an anti-edematous and anti-inflammatory agents in healing of soft tissue injuries, and also in the treatment of arthritis, hematoma, and necrotic tissue (Bartholomew *et al.,* 2003; Dutta and Bhattacharyya 2013; Xie, *et al.,* 2006). Generally, *Ananas comosus* peel has been deployed as animal feed and land fertilizer (Makinde *et al.,* 2011). Antibacterial Activity of *Ananas comosus* wastes has brought about the discovery of new drugs used as food poisoning control and antibacterial (Hikal *et al.,* 2021). (Lubaina *et al.,* 2019) reported the antibacterial potential of ethyl acetate extract of pineapple peel was effective against gram-positive and gram-negative bacterial

strains such as Staphylococcus aureus (ATCC 29213), E scherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Vibrio cholera (MCV09) and Klebsiella pneumoniae (ATCC 700603). Das *et al.*, 2019 reported antioxidant, antibacterial, antidiabetic, and cytotoxicity of *Ananas comosus* peels. Flavonoids and polyphenols present in *Ananas comosus* are more potent in inhibiting gram-positive bacteria (Hikal *et al.*, 2021).

In a recent study done by Luciana *et al.*, 2013, the aqueous Extracts of pineapple peels extracts were tested against engorged females cattle ticks *Rhipicephalus* (Boophilus) *microplus* and also on their larvae. About five different concentrations for aqueous Extracts of pineapple peels extracts (AEPP) were used 500, 250, 125, 62.5, 31.2, 15.6, 7.8 mg/ml. Engorged females were accessed for of survival, oviposition and larval hatching for a period of 24hrs. The LC_{50} and LC_{90} value reported in (AEPP) were 276 and 8691 mg/mL respectively. The most effective concentration for the experiment with engorged females, was 125, 250 and 500 mg/mL: 33%, 48% and 59% for the AEPP. AEPP showed oviposition inhibition of 39.1%, Hatching inhibition of 33.3%, estimated production of 59.4% and Acaricidal efficacy of 59.7%. Luciana *et al.*, 2013 concluded by recommending pineapple extracts as effect against ectoparasite. But AEPP had no efficacy on larvae.

(Rafiki and Nindia, 2022) tested the repellent properties of *Ananas comosus peels* extracts against *Aedes aegypti*. The number of mosquitoes that perched on the hand bait of extract was observed at 10%, 20% and 30% concentration. The hand bait was carried out of cage every 5 minutes in the first hour and every hour for 4 hrs. The observed result showed that average number of mosquitoes perched at a concentration of 10% (18); at a concentration of 20% (16); and at a concentration of 30% (12). Inferring that the higher the concentration of *Ananas comosus extract* the less mosquitoes perched on hand bait. The ANOVA test showed that there was an effect of *Ananas*

comosus peel extract on the number of mosquitoes that perched on hand ($p < 0.05$) significance at 30% with other concentrations of 20% and 10%.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant collection, identification and processing.

Coconut fruits were collected directly from coconut tree in Isihor community, Benin City and pineapple fruits were bought from BDPA maeket Ugbowo Campus University of Benin, Benin. Both samples were identified at the Department of Plant Biology and Biotechnology. The fiber of the coconut fruit were separated from bark but pulling out, and fresh peels of the pineapple were carefully separated from fruit. Both samples were air dried at $27^{O}C$ for 3 weeks in the laboratory. Afterwards, dried samples were taken to the pharmacogonocy department and pulverized to powder using the British milling machine.

3.2 Aqueous and Ethanol extract preparation.

The pulverized individual samples were weighted with a weighing meter. 200grams of powder coconut fiber and 200grams of pineapple peels plant material were actualized. To prepare the aqueous extract, 200grams of the individual powered plant material was dissolved in 750ml of distilled water, and left for 24hrs with constant staring and shaking. While for solvent extraction, 200grams of each plant material was macerated mechanically in 750ml of ethanol in glass jar for 72hrs with constant staring and shaking. Afterwards filtration was carried out to separate filtrate from residue using filter paper, conical flask and funnel. Filtrate passed through a water bath and crucible of controlled temperature of ($70^{o}C$) to produce the crude extract concentration. The crude extract were persevered in a sample bottles in a refrigerator until use.

3.3 Qualitative Phytochemical analysis

The phytochemical constituents of the fiber of *Cocos nucifera* and peels of *Ananas comosus were* analyzed qualitatively with standard procedure described by Keay *et al.*, (1964) and Ejikeme *et al.*, (2014) to determine the presence of secondary metabolites, such as alkaloids, tannins, saponins, flavonoids, steroid and glycosides.

3.4 Larvae collection and culturing

Culex mosquito egg rafts were collected from an abandoned water beaker in the Plant Biology and Biotechnology garden, University of Benin using a deeper. This was later transferred into plastic sample bowls and taken to the insectary laboratory for culturing. A culture medium containing a mixture of tap water and instant yeast was prepared to feed larval. larvae were cultured in medium inside a standard insect box made of simple wood and nets for a period of 6 days at temp (29.2±2^{o}C) and relative humidity (75±4%) until 2nd -3rd instar mosquito larval required for the experiment were bred.

Plate 1: Third instar *Culex* larvae.

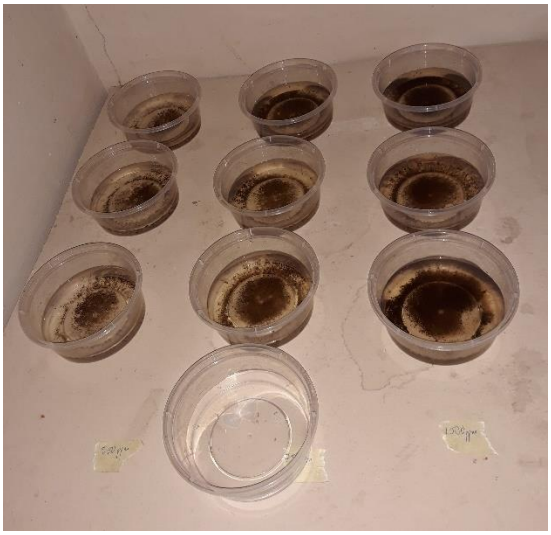
3.5 Preparation of stock solution and concentration.

A sensitive weigh balance was used to weigh 1g, 2g each of aqueous *Cocos nucifera* extract and *Ananas comosus* peels extract. 1g and 2g were also weighed for the ethanoic extract of both *Cocos nucifera* fiber and *Ananas comosus* peels. WHO (2005) Standard procedure was adopted in this study with slight modifications. 2% stock solution was used for each extract of fiber of *Cocos nucifera* and *Ananas comosus* peels. The stock solution of coconut fiber extract and pineapple peels extract was prepared by dissolving 2g of solid extract in 100ml of distilled water. Stock solution of combination of *Cocos nucifera* and *Ananas comosus* was also prepared by dissolving 1g of coconut fiber extract and 1g of pineapple peels extract in 100ml of distilled water.

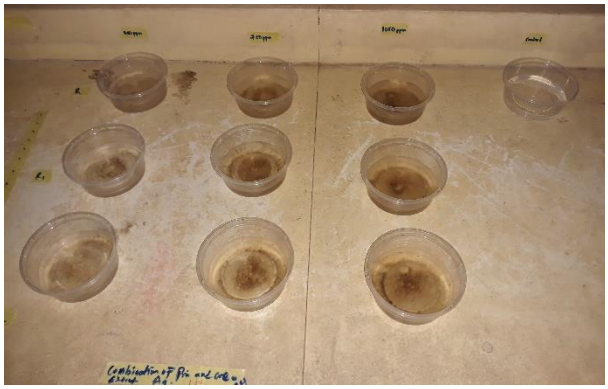
Three concentrations were prepared; 500ppm, 750ppm and 100ppm respectively. For 500ppm, 5ml of the stock solution was added to 95ml of distilled water to make 100ml concentration. For 750ppm, 7.5ml of stock in 92.5ml of distilled water and 10ml of stock solution to 90ml of water for 1000ppm respectively. Each concentration had three replicates and a test control of 100% water (no food material).

3.6 Larvicidal bioassay

10 third instar *Culex* mosquitoes larvae were collected from medium and introduced into test concentration using a pasture pipette for 72hrs at room temperature of 28.2±2°C and a relative humidity of 83±4%. Death and mortality of larvae were observed and recorded at interval of 24, 48, and 72hrs respectively for all test treatments and control. Larvae were considered dead when they showed no movements even when tugged with a pipette and settle at the base of test containers.



a: *Cocos nucifera* test concentration



b: *Ananas comosus* test concentrations

Plate 2: Larval Bioassay.

3.7 Identification of Adult mosquitoes.

5- 10 fourth instar larvae were separated into an inset box before Bioassay and cultured to adults. Active Adults mosquitoes were collected from insect box and transferred into a killing jar with ethyl acetate stuffed tissue paper. Dead adults were then transferred into pendolf bottles packed with silica gel for few hrs. Adult mosquitoes were observed with a dissecting microscope, and identified to be *Culex quinquefasciatus* mosquitos.

3.8 Statistical analysis

The percentage mortality was analyzed using the Statistical Package for Social Scientists (SPSS) 23.0. The LC50 and LC90 values of each plant extract against *Culex quinquefasciatus* larvae was determined after Larval mortality data obtained was subjected to probit analysis on SPSS. Results were transformed at log base 10 at significant value of level of 0.05. Analysis of mortality effects using one-way fractional Analysis of variance (ANOVA), and equal variance assumed the Duncan's Multiple Range test (DMR) to further analyse the significant difference among the various test treatments at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Constituents

Results of the phytochemical analysis of aqueous and ethanolic *Cocos nucifera* fiber, revealed the presence of alkaloids and flavonoids in common strength. Tannins and steroid were present in ethanolic *C. nucifera* fiber but absent in aqueous *Cocos nucifera* fiber which revealed presence of glycosides (Table 1). Phytochemistry of the peels of *Ananas comosus* revealed glycosides and tannins strongly present in both aqueous and ethanolic extracts. Flavonoids, saponins and steroids were absent in aqueous *A. comosus* but showed present in ethanolic *A. comosus* extracts (Table 2).

Table 1: Qualitative phytochemical constituents of *Cocos nucifera* fiber.

Plants	**Tannin**	**Flavonoid**	**Alkaloid**	**Glycosides**	**Saponin**	**Steroids**
Aqueous *C. nucifera* fiber	-	+	++	+	-	-
Ethanolic *C. nucifera* fiber	++	+	++	-	-	+

Key: ++ strongly present; + present; - absent

Table 2: Qualitative phytochemical constituents of *Ananas comosus* peels.

Plants	**Tannin**	**Flavonoid**	**Alkaloid**	**Glycosides**	**Saponin**	**Steroids**
Aqueous *A. comosus* peels	++	-	+	+	-	-
Ethanolic *A. comosus* peels	++	+	++	+	+	+

Key: ++ strongly present + present, - absent

4.2 Effect of concentrations on *Cx. quinquefasciatus* larvae.

Significant difference P<0.05 after 24hrs exposure of the aqueous extracts of *Cocos nucifera* fiber on *Cx. quinquefasciatus* larvae was observed. No significant P>0.05 difference in mortality was recorded at 48hrs of exposure. After continued exposure of *Cx. quinquefasciatus*, at 72hrs a highly significant p<0.05 difference was recorded (Table 3). The Ethanol extract of *Cocos nucifera* showed no significant difference P>0.05 at 24hrs of exposure. At continued exposure of *Cx. quinquefasciatus* to ethanol concentration of *Cocos nucifera* extract there was significant difference P<0.05 after 48hrs of exposure and showed a much more significance P<0.05 after 72hrs of exposure. Larval mortality increased in respect to length of exposure. At 72hrs, the highest mortality was recorded (Table 4).

The aqueous extracts of *Ananas comosus* on *Culex quinquefasciatus*, showed no P value after 24hrs of exposure, although revealed larval mortality of 10% at 1000ppm. At continued exposure of 48hrs, P showed significant <0.05. After 72hrs, a more significant P<0.05 value was recorded (Table 5). Ethanoic *Ananas comosus* extracts showed no significance P>0.05 at 48 hrs. But there was significance P<0.05 at 24hrs of exposure. The mortality difference at continual exposure of 72 hrs. Showed significant p<0.05. Moderate larval mortality effect was recorded with increase in exposure time (Table 6).

The synergistic effect on mortality of Aqueous extracts of *Cocos nucifera* fiber and *Ananas comosus* peels showed significant P<0.05 at 24hrs of exposure. No significant P>0.05 difference following 48hrs of exposure, but after 72hrs of exposure, mortality difference revealed significant P<0.05 (Table 7). Ethanoic extracts of synergy, showed significant P<0.05 throughout 24, 48 and 72 hrs of exposure. Highest significance in mortality was observed after 48hrs exposure time (Table 8).

Table 3: Effect of Aqueous extracts of *Cocos nucifera* fiber against *Culex quinquefasciatus*.

Plant type	**Conc. (ppm)**	**n**	**Mean ± SD (Percentage Mortality)**		
			24hrs	**48hrs**	**72hrs**
Aqueous	0	3	0.00	0.00	0.00
Cocos	500	3	0.00^{b}±0.00(0)	0.06^{a}±0.58(6.67)	1.00^{b}±0.00(10)
nucifera	750	3	0.67^{ab}±0.58(6.67)	1.67^{a}±0.58(16.67)	1.67^{b}±0.58(16.67)
	1000	3	1.33^{a}±13.33(0.58)	1.67^{a}±0.58(16.67)	2.67^{a}±0.58(26.67)
	F-value		6.00	3.00	9.50
	P-value		0.04	0.13	0.01

Table 4: Effect of Ethanol extracts of *Cocos nucifera* fiber against *Culex quinquefasciatus*.

Plant type	**Conc. (ppm)**	**n**	**Mean ± SD (Percentage Mortality)**		
			24hrs	**48hrs**	**72hrs**
Ethanol *Cocos*	0	3	0.00	0.00	0.00
nucifera	500	3	0.67^{a}±0.58(6.67)	1.33^{b}±0.58(13.33)	2.00^{b}±0.0(20)
	750	3	1.33^{a}±0.58(13.33)	2.33^{ab}±0.58(23.33)	2.67^{b}±0.58(26.67)
	1000	3	2.00^{a}±1.0(20.0)	3.33^{a}±0.58(33.33)	4.67^{a}±0.58(46.67)
	F-value		2.40	9.00	26.00
	P-value		0.17	0.02	0.001

Table 5: The Effect of Aqueous extracts of *Ananas comosus* against *Culex quinquefasciatus*.

Plant type	**Conc. (ppm)**	**n**	**Mean ± SD (Percentage Mortality)**		
			24hrs	**48hrs**	**72hrs**
Aqueous	0	3	0.00	0.00	0.00
Ananas comosus	500	3	(-)±0.0(0.0)	0.00^{b}±0.0(0.0)	0.00^{b}±0.0(0.0)
	750	3	(-)±0.0(0.0)	0.67^{ab}±0.58(6.67)	0.67^{b}±0.58(6.67)
	1000	3	(-)±0.0(10.0)	1.33^{a}±0.58(13.33)	2.00^{a}±1(20.0)
	F-value		-	6.00	7.00
	P-value		-	0.04	0.03

Table 6: The Effect of Ethanol extracts of *Ananas comosus* against *Culex quinquefasciatus*.

Plant type	**Conc. (ppm)**	**n**	**Mean ± SD (Percentage Mortality)**		
			24hrs	**48hrs**	**72hrs**
Ethanol	0	3	0.00	0.00	0.00
Ananas comosus	500	3	0.33^{a}±0.58(3.33)	1.00^{b}±0.0(10.0)	1.33^{b}±0.58(13.3)
	750	3	1.00^{a}±1(10.0)	2.00^{a}±0.0(20.0)	2.33^{ab}±0.58(23.33)
	1000	3	1.33^{a}±0.58(13.33)	1.00^{a}±0.58(23.33)	3.33^{a}±0.58(33.33)
	F-value		1.40	13.00	9.00
	P-value		0.32	0.01	0.02

Table 7: The effects combined aqueous extracts of *Cocos nucifera* fiber and *Ananas comosus* *against Culex quinquefasciatus*

Plant type	**Conc. (ppm)**	**n**	**Mean ± SD (Percentage Mortality)**		
			24hrs	**48hrs**	**72hrs**
Aqueous	0	3	0.00	0.00	0.00
Cocos nucifera	500	3	0.00^{b}±0.0(0)	0.33^{b}±0.58(3.33)	0.32^{b}±0.58(3.33)
+	750	3	0.33^{b}±0.58(3.33)	1.33^{ab}±0.58(13.33)	2.00^{a}±0.0(20.0)
Ananas comosus	1000	3	1.33^{a}±0.58(13.33)	1.67^{a}±0.58(16.67)	3.00^{a}±1(30.0)
	F-value		6.50	4.33	12.25
	P-value		0.03	0.07	0.01

Table 8: The effects combined Ethanoic extracts of *Cocos nucifera* fiber and *Ananas comosus* against *Culex quinquefasciatus*.

Plant type	**Conc. (ppm)**	**n**	**Mean ± SD (Percentage Mortality)**		
			24hrs	**48hrs**	**72hrs**
Ethanol	0	3	0.00	0.00	0.00
Cocos nucifera	500	3	0.00^{b}±0.0(0.0)	0.00^{c}±0.0(0.0)	0.67^{b}±0.58(6.67)
+	750	3	0.67^{b}±0.58(6.77)	1.33^{b}±0.58(13.33)	2.33^{b}±1.16(23.33)
Ananas comosus	1000	3	2.33^{a}±0.58(23.33)	3.33^{a}±0.58(33.33)	5.33^{a}±1.53(53.33)
	F-value		19.50	38.00	12.58
	P-value		0.002	0.000	0.007

4.3 Lethal dose concentrations.

At 24, 48 and 72hrs respectively, the lethal concentration of Aqueous *Cocos nuciferia* fiber extracts were LC_{50}: 4043.63ppm, 5161.84ppm, and 1996.30ppm respectively. While LC_{90} Values at 24, 48 and 72hrs were 15978.11ppm, 44647.5ppm and 7552.66ppm respectively. The lethal concentration of Ethanolic *Cocos nuciferia* fiber extracts at 24, 48 and 72hrs, were LC_{50}: 2424.10ppm, 1558.91ppm and 1160.15ppm respectively, while LC_{90} Values at 24, 48 and 72hrs were 9062.24ppm, 5753.94ppm and 3775.93ppm respectively (Table 9).

The Aqueous extract of *Ananas comocous* Peels gave LC_{50} values of 22279.7ppm, 4043.63ppm, and 1969.57ppm at 24, 48, and 72hrs. LC_{90} values were 272866.3ppm, 15678.11ppm and 4485.93ppm at 24, 48 and 72hrs respectively. The lethal concentration of ethanolic *Ananas comocous* Peels extract, At 24, 48 and 72hrs, were LC_{50}: 8676.96ppm, 2342.512ppm, and 1558.91ppm, while LC_{90} Values at 24, 48 and 72hrs were 84888.7ppm, 11494.57ppm and 5752.94ppm respectively (Table 10).

The lethal concentrations of synergy of *Cocos nuciferia* fiber and *Ananas comocous* Peels aqueous extracts at 24, 48 and 72hrs, were LC_{50}: 4584.20ppm, 2751.22ppm and 1996.30ppm respectively. LC_{90} values at 24, 48 and 72hrs were 18113.90ppm, 110071ppm and 2663.96ppm respectively. At 24, 48 and 72hrs, the lethal concentration of ethanolic were LC_{50}: 1262.61ppm, 1136.44ppm, and 983.99ppm respectively, while LC_{90} Values were 1926.67ppm, 1748.55ppm and 1703.40ppm at 24, 48 and 72hrs respectively (Table 11).

Table 9). Lethal concentrations of *Cocos nuciferia* fiber extracts.

Plant type	**Lethal Concentration (ppm)**	**24hrs**	**48hrs**	**72hrs**
Aqueous *Cocos*	LC_{50}	4043.63	5161.84	1996.30
nuciferia fiber	LC_{90}	15978.11	44647.5	7552.66
Ethanolic *Cocos*	LC_{50}	2424.10	1558.91	1160.15
nuciferia fiber	LC_{90}	9062.24	5753.94	3775.93

(Table 10). Lethal concentrations of *Ananas comocous* Peels extracts

Plant type	**Lethal Concentration (ppm)**	**24hrs**	**48hrs**	**72hrs**
Aqueous *Ananas*	LC_{50}	22279.7	4043.63	1969.57
comocous Peels	LC_{90}	272866.3	15678.11	4485.93
Ethanol *Ananas*	LC_{50}	8676.96	2342.512	1558.91
comocous Peels	LC_{90}	84888.7	11494.57	5752.94

(Table 11). Lethal concentrations of synergistic *Cocos nuciferia* fiber and *Ananas comocous* Peels extracts.

Plant type	**Lethal Concentration (ppm)**	**24hrs**	**48hrs**	**72hrs**
Aqueous *Cocos*	LC_{50}	4584.20	2751.22	1996.30
nuciferia fiber + *Ananas comocous* Peels	LC_{90}	18113.90	110071.	2663.96

Ethanol *Cocos nuciferia fiber + Ananas comocous* Peels	LC_{50}	1262.61	1136.44	983.99
	LC_{90}	1926.67	1748.55	1703.40

4.4 Comparative percentage mortality

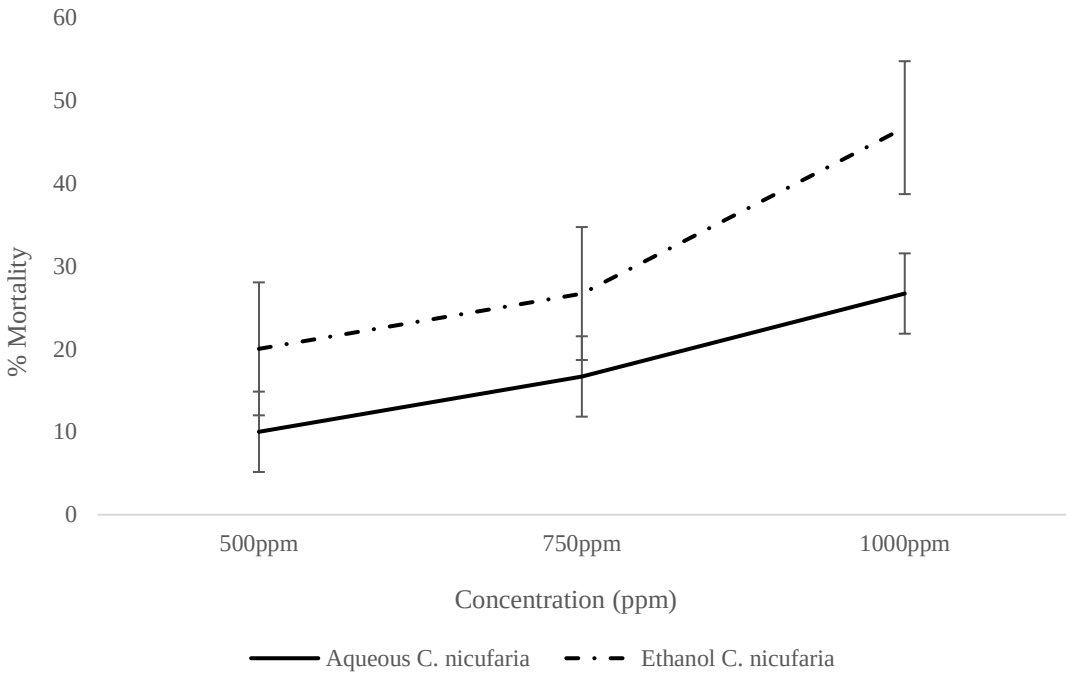


Fig 1: Comparative percentage mortality of aqueous *C. nucifera* and ethanol *C nucifera* on *Cx. quinquefasciatus* after 72hrs exposure.

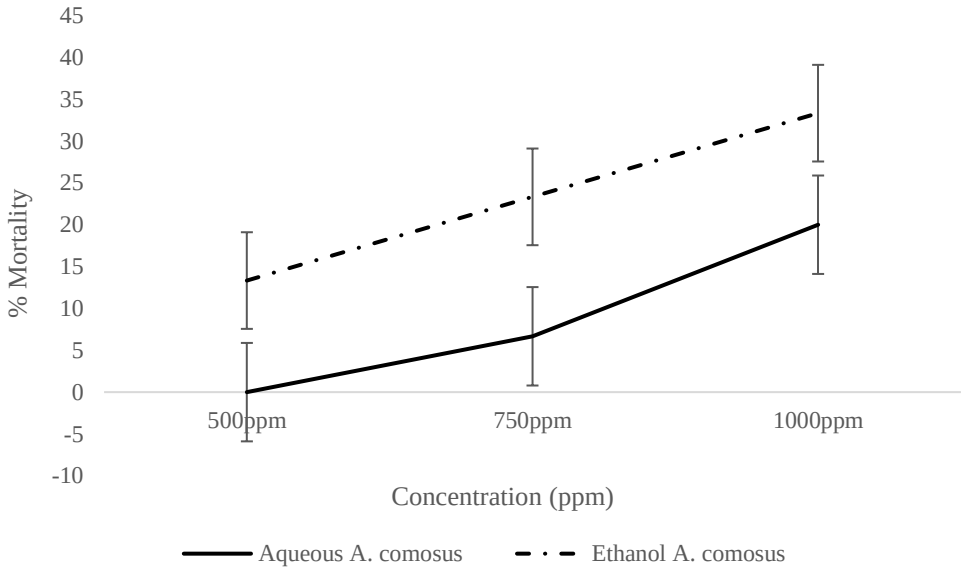


Fig 2: Comparative percentage mortality of aqueous *A. comosus* and ethanol *A. comosus* on *Cx. quinquefasciatus* after 72hrs exposure.

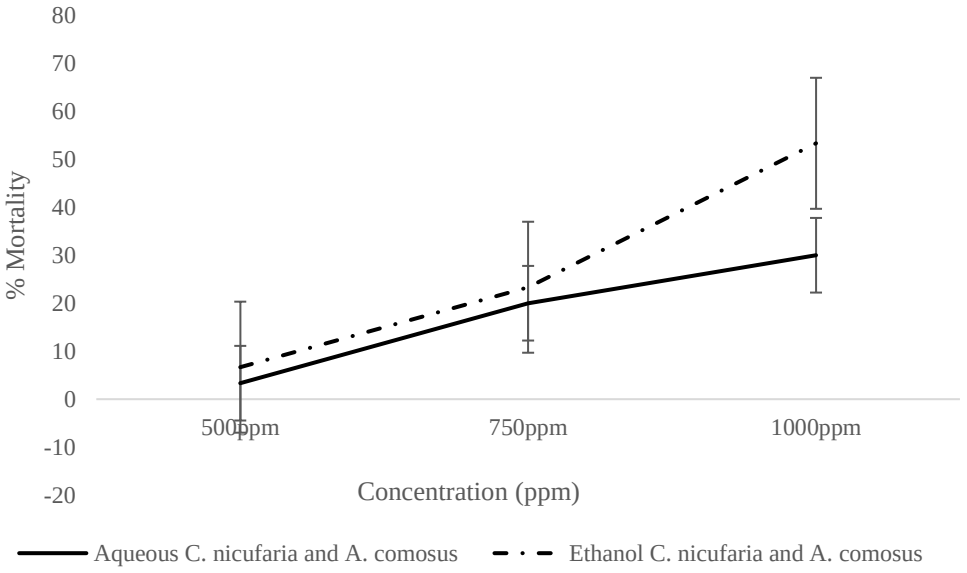


Fig 3: Comparative percentage mortality of combined aqueous and ethanolic *C. nucifera* and *A. comosus* extracts on *Cx. quinquefasciatus* after 72hrs.

CHAPTER FIVE

5.0 DISCUSSION

Qualitative phytochemical constituents of the extracts of *Cocos nucifera* fiber and *Ananas comosus* peels.

The results gotten from the phytochemical screen of Aqueous *Cocos nucifera* fiber extract, revealed presence of flavonoids, alkaloids and glycosides. The ethanolic extract revealed the presence of tannin, flavonoids, alkaloids and steroids. This result obtained was consistent with phytochemical studies reported by Lima *et al.*, (2015), and other secondary metabolites such as protein, starch, terpenoids, and resins were also present. (Dabesor *et al.*, 2017).

The Qualitative phytochemical constituents of aqueous *Ananas comosus peels* extract, revealed the presence of tannin, alkaloid and glycosides, ethanolic extracts of *Ananas comosus peels* showed present for tannin, flavonoids, alkaloids, glycosides, saponins and steroids. Similar results from other studies that has reported alkaloids, flavonoids and saponin showed the most abundant constituent (Rafiki and Yuni, 2022; Dabesor *et al.*, 2017).

Larvicidal effects *of Ananas comosus* peels extract and Cocos nucifera fiber extract on *Cx. quinquefasciatus* larvae.

The highest percentage mortality of *Cx. Quinquefasciatus larvae* recorded at 72hrs of exposure to 500ppm and 1000ppm of Aqueous *Cocos nucifera* fiber extract was 10% and 26.67%. While the ethanolic *Cocos nucifera* fiber showed 20% and 46.67% at 500ppm and 1000ppm respectively. The difference in mortality in 1000ppm of Aqueous *C. nucifera* and ethanolic *C. nucifera* was

20%. This could be as a result of strong presence of Tannans phytochemical in ethanolic *C. nucifera,* which was absent in Aqueous *C. nucifera*. A similar justification was made by Sattar *et al., (*2016) in testing Citrus sinensis against *Cx. quinquefasciatus* larvae, attributed the effectiveness of Citrus sinesis to strong presence of flavonoids, alkaloids, tannins and saponins, inflecting mortality of 100% at 4% concentration for 5 days of exposure. Sattar *et al.,* (2016) addded that if concentrations of extracts are increased, the mortality will also increase. If additional 750ppm was added to 1000ppm of *C. nucifera,* there may have been a resulting increase in mortality.

When aqueous *A. comosus extracts* was tested at concentrations of 500ppm and 1000ppm after 72hrs showed 0% and 20% repectively. The ethanolic *A. comosus extracts* gave 13.33% and 33.33% mortality in 500ppm and 1000ppm. Observed difference in mortality between aqueous *A. comosus and* ethanolic *A. comosus was* 13.3%. This difference could be as a result of stronger presence of alkaloids in ethanolic *A. comosus* than in aqueous *A. comosus.* Although the total mortality recorded in *A. comosus* was not satisfactory enough to recommend *A. comosus.* The highest LC_{50} and LC_{90} values were observed in *A. comosus.* Luciana *et al.,* (2013) preferred the bromelain extracts of *A. comosus* to aqueous *A. comosus* against *heamonchus controtus.* Due to low effect and reported high LC values in aqueous extracts (Hordegen *et al.,* 2006). The peels of *A. comosus*

A. comosus and C. nucifera aqueous and ethanolic extracts were mixed together in 1:1.Same way different active chemicals present in synthentic insecticides are mixed for synergistic effect. The combined aqueous showed percentage mortality of 3.33% and 30% at 500ppm and 1000ppm respectively, while the ethanolic synergistic extracts gave 6.66% and 53.33% percentage mortality at 500ppm and 1000ppm respectively. it was observed across all test plants extracts that the

ethanolic extracts showed higher mortality percentage that those of Aqueous, this could be attributed to the polar bonds present in ethanol which is more active in extracting plant metabolites, making them more potent as bio insecticides against targeted species. The total observed performance of combined ethanolic *A. comosus and C. nucifera* on *Cx. Quinquefasciatus* larvae proved promising. It was observed through all plant tests, that at 750ppm, there was a trend change or interruption in mortality difference. No reported work has be done to access the larvicidal potential of *Ananas comosus* and *C. nucifera* against *Cx. Quinquefasciatus* larvae.

Conclusion

The Ethanoic extract of *C. nucifera* and *A. comosus* showed more efficacy against 3rd instar *Cx. quinquefasciatus* larvae than the Aqueous extracts. Results showed a fair larvicidal mortality on the target mosquito species in order of; combination of *C. nucifera* and *A. comosus, C. nucifera extract and A. comosus* extract. *C. nucifera* fiber and *A. comosus* peels. The combined ethanolic extract of *C. nucifera* and *A. comosus* can be considered a potential larvicide.

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