



## Evaluation of Bio-active Ingredients of *Arica papaya* and *Azadirachta indica* on Development and Reproduction of *Bemisia tabaci* (Homoptera: Aleyroididea)

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**Abstract:** *Bemisia tabaci* is a cosmopolitan pest affecting a wide range of crops including but not limited to horticultural and ornamental crops. Damage is caused through direct feeding on the phloem sap of host crops thereby inducing physiological disorders and also serving as vector causing viral diseases in insect population. Both forms of damage have direct consequences on crop production, productivity leading to drastic reduction of economic values of the crops and lowering of smallholder farmers incomes. Management of *B. tabaci* in Sierra Leone has been dominated by the use of broad-spectrum chemical pesticides, though having the intrinsic feature of reducing the pest population below the economic injury level, yet their usages are associated with adverse effects on the environment, non-targets pests such as natural enemies and development of resistant genes. Equally important, cost and affordability of pesticides for large scale applications are being considered constant economic burden to smallholder farmers. These challenges have prompted to seek alternative substitutes to broad-spectrum chemical pesticides, with a paradigm shift of the use of plant derived bio-active ingredients significant from plants and fungi serving as bio-pesticides. These bio-pesticides have proven to be quite useful in managing sap sucking insects like *B. tabaci* though their abilities have not been fully exploited in under Sierra Leonean circumstance. This investigation aimed at determining the impact of bio-active ingredients of Neem and Papaya on the reproduction and development of *B. tabaci* under controlled laboratory conditions. Concentrations of the active ingredients of *A. papaya* and *A. indica* were prepared and serially diluted at different concentrations and assayed against eggs and developmental stages of *B. tabaci* in bioassay cages and maintained under controlled laboratory conditions of  $25 \pm 2^\circ\text{C}$  and photoperiodism of 12:12 (L:D). No significant impact % hatchability ( $p=0.0001$ ) as  $> 60\%$  of eggs hatched into crawlers, however larval mortality was dose-dependent, larval mortality was significantly impacted and varied with age of immature in the order 2nd instars  $> 3^{\text{rd}}$  instars  $>$  pupa at ( $P < 0.0001$ ).  $LT_{50}$  values varied across developmental stage, 2nd instars required 2.54 and 4.3 days to inflict 50% mortality in population treated with Neem and Papaya respectively. Larval mortality was dose-dependent with mortality proportionally increasing with increased in concentration of the active ingredients. The  $LC_{50}$  values strongly correlated with developmental stage and concentration in the order 2nd instars  $< 3^{\text{rd}}$  instars  $<$  pupa for neem and papaya extracts respectively. Moreover, the extracts impacted the population dynamics of *B. tabaci* as the population parameters were significantly influenced by the toxicity of the extracts suppressing the growth and population parameters as compared to the control. Comparatively, the neem extracts significantly impacted the demographic parameters  $R_0$ ,  $\lambda$ ,  $r_m$  13.66, 1.103, 0.018 as compared to *A. papaya* extract 18.97, 1.106 0.101 and control 23.56 1.130, 0.123 respectively. The importance and potentials of *A. indica* and *A. papaya* as effective bio-pesticides for sustainable management *B. tabaci* were well highlighted and discussed.

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**Keywords:** Bemisia tabaci, Archicrinictata indica, Arica papaya, Bio-pesticides, demoragrphic parameters, whiteflies

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## INTRODUCTION

*Bemisia tabaci* (Gennadius), commonly known as the Sweet potato whitefly, is a significant economic pest of vegetable crops worldwide and a highly polyphagous species, with a wide host range attacking plant species across 74 families, including vegetables, ornamentals and fruit tree crops [1][3]. *B. tabaci* is considered one of the most destructive insect pests in vegetable production systems causing both direct and indirect damage to vegetable crops. Direct damage includes sap removal where nymphs and adults feed on plant sap by inserting their piercing-sucking mouthparts into the phloem of plant stems and leaves [2] [4] [5]. According to [1] [2] [6], this manner of feeding can lead to weakening and early wilting of the plant leading to a reduced plant growth rate and yield, leaf yellowing, drying, and premature dropping, and under severe circumstances seedling death and stunting [3][4]. Furthermore, it has been proven that whiteflies can inject enzymes that disrupt plant physiology leading to irregular fruit ripening in tomatoes and silverleaf in squash [3] [5]. Indirect damage has been observed in many instances where *B. tabaci* is observed to excrete honeydew, a sugary substance that supports the growth of sooty mold [3][4] having a tendency of reducing photosynthesis by blocking sunlight leading to a reduced plant vigour causing chlorosis resulting in an uneven ripening and physiological disorders with a resultant effect of lessening the market value of the plant. *B. tabaci* does not only cause havoc to the physical parts of plants but is found to transmit viral diseases over 100 plant viruses [8][10] which can cause significant yield losses, ranging from 20% to 100% under heavy severe situation and under heavy infestation can lead to total collapse of the entire plant. [7][10]. Some of the most damaging viruses transmitted by *B. tabaci* including but not limited to Tomato Yellow Leaf Curl virus (TYLCV), Tomato Chlorosis Virus (ToCV), Cucurbit Yellow Stunting Disorder Virus (CYSDV), Cucumber Vein Yellowing Virus (CVYV), Squash vein yellowing virus (SqVYV) [9] [11], bean golden mosaic virus (BGMV) [9] [10] among others. These viral infections further emphasize the aggressiveness of the pest that warrants greater attention. Economic impact has been strongly reported in West Africa, where cassava mosaic and cassava brown streak diseases, transmitted by *B. tabaci*, result in a 50% loss of cassava production and annual losses of significant economic consequences. Infestations can also lead to stunting, yellowing, mottling, and stem blanching in leafy vegetable crops, making them unmarketable.

Management of *B. tabaci* has been mostly associated with the use of broad-spectrum chemical pesticides. Though these chemicals have a significant impact on the mortality of the pest by reducing the pest population below the economic injury levels, however the control is quite temporal with significant side effects. The phenomenon of pest resurgence is the ability of pest population to dramatically increase beyond the economic injury level as the overuse of insecticides leads to the evolution and adaptation of pests, increasing the prevalence of pest resurgence and pesticide-resistant species are points to note due to frequent use of pesticides leading to the evolution of *B. tabaci* resistant population has been demonstrated in fields and have observed to display high resistance to synthetic chemicals like methamidophos and chlorpyrifos due to their long-term application [12] [13]. Equally important broad-spectrum insecticides kill both beneficial insects and pests including

predators and parasitoids that help control *B. tabaci* populations under natural conditions . Among the most commonly use is the organophosphates, carbamates, and pyrethroids are particularly harmful to beneficial species [12][13] with an impact leading to the reduction of natural enemies with a consequent effects of pest resurgence and secondary pest outbreaks [12] , where predators and parasitoids are often more susceptible to pesticides than plant-feeding insects because plant-feeding insects may possess detoxification mechanisms produced by plants [13], despite the use sub-lethal doses . Additionally, the use of broad-spectrum pesticides is found to impact environmental contamination and non-target organism. [23] indicated that over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields contaminating the soil, air, and water with a significant consequence leading to the decline of water quality and under severe conditions, the contaminated water through erosion is drained into standing body leading to eutrophication. Equally important , pesticides can impact soil microorganisms, which are essential for nutrient cycling and organic matter decomposition [8] with a consequence effect the persistence of insecticides in the soil can lead to bioaccumulation in soil organisms, plants, animals, and humans through the food chain. Pesticides can also have indirect effects by reducing the number of plants and insects that provide food for beneficial insects [12] [13]. The side effects with significant impacts are a wake-up call to shift to an alternative in the management of *B. tabaci* in regions that solely rely on the use of broad-spectrum chemical pesticides.

Among the numerous alternatives, the management of *B. tabaci* using selective insecticides focuses on minimizing the impact on beneficial insects while effectively controlling the pest. The use of selective pesticides where the active ingredients target either the morphological and physiological functions of the insects without environmental interference has been as a critical and plausible method of pest management . Among such alternatives is the use of Insect Growth Regulators (IGRs) such as Methoxyfenozide that targets the hormonal processes in insects, disrupting molting and development processes of the insects [14] [16] [17]. It has been reported that neonicotinoids provides good efficacy against *B. tabaci* [16] [18] while being less harmful to natural enemies compared to other neonicotinoids. In a similar trend thiamethoxam is a selective pesticide that has proven to be effective against *B. tabaci*, but its use should be limited due to potential resistance development [20] [21] [22]

The use of botanical insecticides such as Azadirachtin and Pyrethrins disrupts feeding and reproduction, with minimal impact on non-target organisms has be well recognized as a plausible natural means of pest management strategy [16]. The neem leaves contain azadirachtin, a potent insect growth regulator that disrupts the life cycle of pests by inhibiting larval development and reducing fecundity and have been proven to be quite effective against a wide range of pests, including aphids, whiteflies, and caterpillars, neem acts as both a repellent and a growth disruptor. This broad-spectrum activity is quite effective against various insect pests without adversely affecting beneficial insects, making it suitable for integrated pest management (IPM) strategies [17][18] [19] . Moreover, the neem leaves are found to contain antifeedant properties that have been characterized and significantly underscored to deter feeding in many insect species, reducing damage to crops lowering pest populations over time [17][18] , and their use can lower the reliance on synthetic pesticides, promoting environmentally friendly agricultural practices further

improvement. A striking property of neem leaves is that it is found to enhance soil health by promoting beneficial microbial activity, further supporting plant health and resilience against pests [16] [17] [18].

The papaya leaves on the other hand contain compounds such as papain and carpaine [14] [15] which have demonstrated insecticidal effects against various pests, including whiteflies and aphids, enhance the nutritional status of plants, making them more resilient to pest attacks while also promoting overall plant health. According to [14] incorporating papaya leaves into crop rotations or as mulch can help suppress pest populations naturally, contributing to sustainable agricultural systems. Extracts from papaya leaves can be formulated into biopesticides, providing an alternative to chemical pesticides and reducing environmental impact and much has been observed that the strong smell of papaya leaves can act as a natural repellent, deterring pests from infesting crops. Both neem and papaya leaves offer effective, eco-friendly alternatives for managing insect pests in horticultural crops [14] [15] [18]. Their use supports sustainable agricultural practices, promotes plant health, and helps maintain biodiversity in agricultural ecosystems. Utilizing these natural resources can significantly reduce reliance on synthetic pesticides, contributing to healthier crops and environments [11] [15].

In Sierra Leone, neem trees and papaya fruit trees are quite common growing widely and in some instances are cultivated under agro-forestry schemes in greater part of the country, thus can be considered as a potential source for developing bio-pesticides to manage insect pests like *B. tabaci* that affects a wide range of crops notably vegetables and other horticultural crops [personal communication].

Investigating life table parameters is crucial for effective insect pest management because it provides a comprehensive understanding of a pest's population dynamics, enabling the development of targeted and sustainable control strategies. Life tables offer detailed insights into various demographic parameters such as developmental time, fecundity, survival rate, and mortality factors, which collectively determine a pest's population growth potential [33].

It has been indicated by [33] that in many insects, the mortality rate is characteristic of the stage and is not uniform for all the developmental stages; the mortality rate of early and late stages is often higher than those of intermediate stages [33] [24]. Knowledge of the number of immature stages and the mortality factor affecting on each stage may assist in the timing of pest management strategies [33] [35]. More in the study of the effect of mortality factor such on different of insect pest, the fecundity table is an invaluable technique which helps in quantitative and qualitative evaluation of natural enemies that provides convenient method for recording and accounting for population changes in the life cycle of insects. In addition, the overall effectiveness of natural enemies acting on various stages may greatly influence the demographic parameters of insect evaluation [33] [34].

[34] proposed that the use of life table parameters is a strategic evaluation method to obtain insight on population dynamics, and has been opined that this method can serve as an initial step in a strategic and sustainable pest management effort. There is a need to identify and measure the impact of botanical extracts as bio-active ingredients on various developmental stages which can be measured in terms of survival rate, mortality rate, fecundity rate, development time to determine the nature of pest. The fecundity rate is

the number of offspring or eggs that a female insect can produce in its life span thus providing its ability to contribute to its population growth and dynamics, thus is an invaluable instrument for measurement [36][38]. Increased fecundity is much associated with rapid population increase and potential outbreak. The survival rate is another key criterion that provides valuable measures of the ability to thrive taken in consideration that survival rate of *B. tabaci* is a key factor that increases the population size and factor such as mortality factors such as natural enemies are observed to significantly impact survival rates, hence prolonging the insect life span and promoting population dynamics of the insect [13]. Furthermore, it is imperative that identifying key mortality factors is essential for developing targeted management strategies [34] [35]. The combination of survival rates, mortality rates can lead to the determination of intrinsic rate of increase and finite rate of increase which are used as indicators as population growth potential [37]. This information is useful to make a reference as whether the population is either increasing or decreasing. An interference factors like bioactive ingredients derive from plants are quite useful in the management of horticultural pests [24].

This research focuses on the investigation of bio-active ingredients derived from *A. papaya* and *A. indica* which are commonly found growing in diverse ecological zones in Sierra Leone and easily accessible. The active ingredients of these plants are extracted, and solutions prepared at different concentrations and tested against developmental stages of *B. tabaci* to determine the mortality and survival rates for the computation of life table parameters of *B. tabaci*. The study further endeavours to quantify  $LT_{50}$  and  $LC_{50}$  as parametric indices to determine the efficacy of the active ingredients with respect to kinetics and toxicity against *B. tabaci* population. This study therefore is considered as a preliminary effort to determine the potentials of these plants extract as ideal candidates for development of bio-pesticides for the management of *B. tabaci*.

## **MATERIALS & METHODS**

### **Preparation of Stock Solutions of Neem and Papaya**

Fresh leaves of neem and papaya were collected from healthy neem and pawpaw, the leaves were thoroughly washed with distilled water to remove the dust and contaminants. Thereafter the leaves were air-dried at room temperature to avoid excessive moisture. About 2.5g of clean dried leaves were grind into a fine paste using iron casted mortar in order to increase the surface area for efficient extraction of the active ingredients. Thereafter 100% of 50mL of ethanol was added to the grinded paste to form a slurry in a conical flask, and then placed in a shaky incubator and then confined at 37°C. The slurry solution is stirred intermittently every 2 hours to ensure uniform extraction, and the resultant solution is left for 12-16 hours to permit the evaporation of ethanol concentrating the extract into a dry or semi-dry powder residue containing the bioactive compounds. The resultant powder is weighed and suspended in the dimethyl sulfoxide (DMSO) to achieve a stock concentration of 80 mg/mL. The resultant solution is filtered thoroughly through a syringe filter to sterilize and remove any particulate matter or debris to form the stock solution which is stored at -20°C in aliquots to prevent degradation. The stock solution is maintained and served as the base for diluting to test concentrations.

## Preparation of Test Concentrations

For the dose-response investigation, five concentrations were prepared from the stock solution as indicated:  $0.2\text{mg/mL}^{-1}$ ,  $0.4\text{mg/mL}$ ,  $0.6\text{mgmL}^{-1}$ ,  $0.8\text{mg/mL}^{-1}$  and  $1\text{mg/mL}^{-1}$  respectively. The control was made up of distill water with Tween 80 solution to investigate the dose- mortality response of bioactive ingredients of Neem and papaya.

## Effects of Bio-active Ingredients of Neem and Papaya on Egg Hatchability

The effects of bio-active ingredients on egg hatchability and larval development were determined on detached leaves poinsettia plants confined in petri-dishes. For a homogenous and cohort egg production, a micro cage was attached to the lower surface of the leaves and about 3-5 *B. tabaci* undetermined sex adults were released with aspirator into the micro cage for a period of 2 days for egg production. Thereafter, a cohort of 30 eggs were examined under the microscope and their positions demarcated and marked with indelible ink and later sprayed to run off with five different concentrations of neem and papaya and a control treated with 0.05% Tween 80. From previous knowledge on egg development on poinsettia plants, the leaves were detached 2 days prior to hatching and placed in petri-dish aligned in the bottom with moist filter paper. In addition, water is added daily to prevent desiccation, and then incubated under standard experimental conditions of  $25 (\pm 2^\circ\text{C})$  and  $75 \pm 10$  RH. The crawlers which are the newly hatched larvae were recorded and their positions marked with indelible ink after settling on the leaves. The development and survival rates of these crawlers were monitored for 4 days based on preliminary insight on this host plant.

## Dose-Response Relations: Lethal Time ( $LT_{50}$ ) and Lethal Concentration ( $LC_{50}$ )

### Determination of $LT_{50}$

For determination of  $LT_{50}$ ,  $1\text{mgmL}^{-1}$  of the bioactive ingredients of papaya and neem were assayed against three developmental instars *B. tabaci*  $n=30$  for each batch (2<sup>nd</sup>, 3<sup>rd</sup> instars and the pupa stage) respectively. The poinsettia host plants bearing the immatures were dipped in the stock solution briefly for 2 mins and then confined in a micro clip cage underneath the poinsettia plants and maintained in bioassay cages  $60 \times 60 \times 60$  under controlled laboratory conditions. Dead/live instars were monitored daily under stereomicroscope for twelve (12) consecutive days. The cumulative mortality during this period was computed and subjected to Abbot mortality correction. The corrected mortality values were subjected to probit analysis by transforming mortality values to proportion of dead instars ( $p$ ) and linearly regressed against  $\text{Log}(T)$  at  $p=5$  (50%) to determine  $LT_{50}$  which is the time required to kill 50% of the tested immatures. For this investigation, the protocols for each developmental stage are similar and maintained under the same experimental conditions as mentioned above.

### Determination of $LC_{50}$

Determination of  $LC_{50}$  was carried on the nymphal 2<sup>nd</sup>, 3<sup>rd</sup> instars and pupa stage of *B. tabaci* reared on poinsettia plant. Based on preliminary knowledge on development of *B. tabaci* immatures on this host plant, the rearing dates were synchronized to enhance monitoring

of their development alongside each immature stage. For monitoring of each immature developmental stage, the positions of cohort of ( $n = 30$ ) immatures of 2<sup>nd</sup>, 3<sup>rd</sup> instars and pupa stage on the poinsettia plants were demarcated and exposed to various concentrations: 0.2mg/mL<sup>-1</sup>; 0.4mg/mL<sup>-1</sup>, 0.6mg/mL<sup>-1</sup>, 0.8mg/mL<sup>-1</sup> and 1mg/mL<sup>-1</sup> whilst the control treatment was sprayed with 0.05% Tween 80. Thereafter, the inoculated poinsettia leaves bearing the immatures were monitored daily under microscope to adult emergence. The experiment was replicated five times.

### Bioassay on Life Table Parameters

For investigation of development period, survival and mortality rates leading to adult emergence, the poinsettia leaves bearing eggs of *B. tabaci* were inoculated with various concentrations 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL and 1mg/mL bioactive ingredients of neem and papaya and 0.05% Tween 80 serving as control treatment. Five inoculated leaves were carefully selected and the positions of the 5 eggs from each leaf demarcated with indelible inks making up ( $n = 25$ ) for monitoring to adult emergence. The demarcated leaves bearing the eggs were identified and confined in bioassay cages for subsequent development. The number of eggs hatched were carefully monitored to adult emergence taken into consideration the mortality and survival rates of nymphs. At the red-eye pupal stage, the experimental leaves are detached from the main host plants and the pupal positions identified and marked with inedible ink and then confined petri-dish covered with nylon cloth for adult emergence.

### Effect of Bio-active Ingredients on the Fecundity and Longevity of *B. tabaci*

The sex ratio of newly emerged adults *B. tabaci* were separated into sexes and maintained for 2 days thereafter 10 female adults were selected and individually clipped to the under-surface part of the poinsettia plants then briefly submerged at different concentrations 0.2mg/mL<sup>-1</sup>, 0.4mg/mL<sup>-1</sup>, 0.6mg/mL<sup>-1</sup>, 0.8mg/mL<sup>-1</sup> and 1mg/mL<sup>-1</sup> and the control treated with 0.05% Tween 80 for 10 minutes and then confined in bioassay cages under aforementioned experimental conditions. The number of eggs laid per day was carefully monitored until the last female died to determine fecundity and longevity of the female adults.

### Life Table and Demographic Parameters Models

The potential effects of bioactive ingredients from the test materials at population level, data of immatures survival, adult survival and female reproduction were used to make a cohort life table and fecundity scheduled according to [37], whilst life and fertility tables were constructed and calculated according to [36]. the death  $q_x$  and survival rates  $S_x$  and the probability of surviving from birth to age  $X$  ( $l_x$ ) for every mature stage were computed. The intrinsic rate of population increase  $r_m$  was calculated using the method of [37] [38]:

$$R_0 = \sum l_x m_x$$

$$T = \sum x l_x m_x / \sum l_x m_x$$

$$R^m = \ln R_0 / T$$

$$\lambda = \exp(r_m)$$

$$GT = \ln (Ro) / r_m$$

$$DT \ln(2) / r_m$$

The  $L_x$  is the survivorship at the corresponding time ,  $M_x$  is the number of female eggs laid per day per female (egg female<sup>-1</sup> day<sup>-1</sup>)

The gross reproductive rate =  $\Sigma M_x$

The net reproductive rate  $R_o$  is the mean number of female progeny by a single female during its mean life span. This parameter expresses per generation growth rate of the population and is related to the discrete daily growth rate . The finite rate of increase was calculated by the equation.

The Generation Time (GT) which is equivalent to the mean period elapsing between birth of the parents and birth of the of the offspring , and the Double Time (DT) is the is defined as the time required for the population to double in size [39] .

### Data Collection and Analysis

The effect of bio-active ingredients on egg hatchability which is expressed as the number of eggs hatched and larval survival and development were computed and mean values compared with LSD to detect any significance difference among concentrations ( $p=0.0001$ ) level of significance. One-way analysis of variance test was applied to the data to determine the effects of bioactive concentrations on survivorship and development of the immatures at ( $p=0.0001$ ) level of significance.  $LC_{50}$  and  $LT_{50}$  were determined, the percentage mortality of each stage was computed and subjected to Abbot's mortality correction factor prior to statistical analysis. The mortality values were log transformed and linearly regressed against time and data fitted to Linear model to compute  $LT_{50}$  and  $LC_{50}$  respectively. Analysis of variance test F-test ( $p=0.0001\%$ ) was carried out for any significant variation in mortality due to concentrations and time respectively. Means were computed and compared to by Newman-Keule means of comparison at ( $p=0.0001$ ) level of significance

Life table and Demographic Parameters - The survival and development of *B. tabaci* female adults were recorded at different concentrations, the daily number of eggs laid and the life span of the female adults were recorded to calculate the fecundity and longevity at different concentrations. The eggs laid were subjected to one-way of variance (ANOVA) test at ( $p=0.0001$ ) , means separated and compared to detect any significant variations.

## RESULTS

Results on the effect of various concentrations of the active ingredients of Papaya assayed on a batch of *B. tabaci* eggs ( $n=30$ ) to determine hatchability and larval development are presented in (Table 1a). Results indicated that the active ingredient concentrations did not significantly impact eggs hatchability ( $p>0.0.001$ ) as % hatchability was observed to be over 65% irrespective of extract concentrations. Results further showed no significant difference ( $P<0.00015$ ) in % hatchability of eggs treated with abstract concentrations except at the highest concentration 1mgmL<sup>-1</sup> where the % hatchability was 66% (Table1a). In contrast, larval mortality however indicated dose-dependent with mortality increasing with increased in concentrations of the active ingredients (Table 1a). At the lowest concentration of



0.2mgmL<sup>-1</sup>, larval mortality was recorded as 39.56. For batches treated with the highest concentration 1mgmL<sup>-1</sup> of papaya extract and Tween 80, the mortality values were 69.09 and 15.34 respectively ( $p < 0.0001$ ) (Table 1a)

**Table 1a: Effect of Bio-active conc. of Papaya on egg hatchability and nymphal development**

Treatment (Conc.) (mgmL <sup>-1</sup> )	Mean (Hatched Eggs)	% hatchability	% Larval mortality
0.2mgmL <sup>-1</sup>	23.09 a	83.33	39.56 a
0.4 mgmL <sup>-1</sup>	21.33 a	73.33	42.46 a
0.6mgmL <sup>-1</sup>	24.55 a	80.00	65.10b
0.8mgmL <sup>-1</sup>	22.75 a	89.16	67.21 b
1.0m/mL <sup>-1</sup>	20.21 b	66.66	69.09b
Ck (Tween80)	26.89 a	87.21	15.34 c

Means were separated by LSD at ( $p=0.0001$ ). Means in the same column with the same letters are not significantly different. A cohort (n=30) eggs were treated per concentration whilst the control Ck was treated with Tween 80.

**Table 1b: Effect of Bio-active conc. of Neem on egg hatchability & nymphal mortality**

Treatment (Con.) (mg/mL)	Mean of (Hatched Eggs)	% Hatchability	% Larval mortality
0.2mgmL <sup>-1</sup>	23.13a	77.10a	45.34a
0.4 mgmL <sup>-1</sup>	23.87a	79.56a	47.69b
0.6mgmL <sup>-1</sup>	24.34a	81.13 a	60.01c
0.8mgmL <sup>-1</sup>	23.88a	79.57a	68.34c
1.0 mgmL <sup>-1</sup>	23.74a	79.13a	70.94c
Ck (Tween80)	24.35a	81.16a	9.09d

Means were separated by LSD at 0.1% level of significance ( $p=0.0001$ ). Means in the same column with the same letters are not significantly different. A cohort (n=30) eggs were treated per concentration whilst the control Ck consist of Tween 80.

Results of batch (n=30) eggs were assayed with concentrations of neem extract are presented in (Table 1b). Results of % hatchability were significant with over 75% of the eggs hatched to larval irrespective of concentrations with no significance difference between treated and untreated eggs (Table 1b). However, results on % larval mortality varied with increased in extract concentrations with mortality, the lowest and highest concentrations were recorded as 45.34 and 70.94 % respectively. The larval population treated with Tween indicated lowest % mortality value of 9.09 ( $P < 0.09$ ). In comparison of the impact of two extracts papaya and neem on larval mortality at lowest concentration 0.2mgmL<sup>-1</sup>, % larval

mortality values were 39.56% and 43.34% whilst at the highest concentrations  $1\text{mgmL}^{-1}$ , % mortality was 67.71% and 70.94%. The % mortality values for the Ck for larval populations treated with control treatment Tween 80 were 15.34% and 9.09% respectively.

Results on the impact of papaya and neem extracts on accumulative mortality of 2<sup>nd</sup>, 3<sup>rd</sup> and pupa developmental stages are presented in (Table 2a&2b). The impact of Papaya extract on percentage cumulative mortality values was dose-dependent showing variation in mortality as the conc. of the extract were increased. At the lowest conc of  $0.2\text{mgmL}^{-1}$ , the percentage cumulative mortality for 2<sup>nd</sup>, 3<sup>rd</sup> and pupa instars were 40.54%, 38.44% and 6.54% respectively, whilst at the highest conc  $1\text{mgmL}^{-1}$  a similar trend with values of percentage cumulative mortality were 63.34%, 50.73% and 42.53% were for the respective instars (Table 2a). A similar trend in cumulative values was observed when the immatures were treated with Neem extracts. At the lowest conc, the % cumulative mortality values for 2<sup>nd</sup>, 3<sup>rd</sup> and the pupa stage were 49.33%, 39.89% and 15.98% respectively indicating a decrease in % cumulative mortality values across the developmental stages. Similar trend was also observed at highest conc  $1\text{mgmL}^{-1}$  accounting for 63.34%, 59.73%, 42.33% for 2<sup>nd</sup>, 3<sup>rd</sup> and pupa stage respectively (Table 2b). The impact of neem extract mortality of the immatures were high that than immatures treated with papaya extract for targeted developmental stages (Table 2a& 2b).

**Table 2a:** Effect of Bio-active ingredient of Papaya on cumulative mean mortality of *B. tabaci* immature stages (Nymphal and Pupal Stage)

Treatment (Conc.) ( $\text{mgmL}^{-1}$ )	B. tabaci Instars		
	2 <sup>nd</sup>	3 <sup>rd</sup>	Pupa
$0.2\text{mgmL}^{-1}$	40.54a	38.44a	6.56a
$0.4\text{ mgmL}^{-1}$	42.55a	40.34b	26.09b
$0.6\text{ mgmL}^{-1}$	58.17b	53.56c	39.56c
$0.8\text{ mgmL}^{-1}$	59.21c	56.89d	40.31c
$1\text{ mgmL}^{-1}$	63.34c	59.73e	42.53d
CK	7.45	3.21	0.89

Means were separated by LSD at 0.1% level of significance ( $p=0.0001$ ) . Means in the same column with the same letters are not significantly different. A cohort ( $n=50$ ) immatures were treated per concentration

**Table 2b:** Effect of Bio-active (Neem Extract) on cumulative mean mortality of *B. tabaci* immature stages ( Nymphal and Pupal Stage) maintained for 8 Days under laboratory conditions

Treatment (Conc.) ( $\text{mgmL}^{-1}$ )	B. tabaci Instars		
	2 <sup>nd</sup>	3 <sup>rd</sup>	Pupa
$0.2\text{mgmL}^{-1}$	49.32a	35.89a	15.98a
$0.4\text{ mgmL}^{-1}$	57.56b	42.44b	31.37b
$0.6\text{ mgmL}^{-1}$	59.46bc	44.67c	43.67c

0.8 mgmL <sup>-1</sup>	61.73d	48.88d	44.95cd
1 mgmL <sup>-1</sup>	69.09de	53.61e	56.67d
Ck (Tween80)	06.89	3.55	2.37

Means were separated by LSD at 0.1% level of significance ( $p=0.0001$ ). Means in the same column with the same letters are not significantly different. A cohort ( $n=50$ ) immature was treated per concentration whilst the control Ck batch treated with Tween 80

Effect of Papaya (extracts) of different concentrations were assayed against *B. tabaci* reared on poinsettia plants and maintained under standard laboratory conditions, and results on development period for various stages are presented in (Table 3). The incubation period for the of eggs reared on plants treated with Neem and Papaya concentrations at 1mg/mL were 5.33 and 5.11 respectively (Table 3a). Results indicated no significant difference in development period for treated immatures at ( $p=0.0001$ ), the duration period i.e. life span (egg-adult) for *B. tabaci* batches treated with 1mgmL<sup>-1</sup> concentrations of Neem and Papaya are 22.29 and 21.67 days respectively. The development period for the various stages under control treatment were not significantly different ( $p=0.0001$ ) level of significance from the treated cases (Table 3a).

**Table 3: Comparative Effect of Neem & Papaya extracts on Development Period (Days) of *B. tabaci* reared on poinsettia plants (maintained under laboratory conditions of  $25\pm 2$  °C and  $75\pm 10$  Relative Humidity)**

Treatment	Egg	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	Pupa	Egg-Adult
1mgmL <sup>-1</sup> of Neem Conc	5.33a	3.91a	2.28a	4.37a	3.46a	4.01a	22.29a
1mgmL <sup>-1</sup> Papaya	5.11a	3.21a	2.34a	4.05a	3.22a	3.98a	21.67a
Tween 80 Ck	6.98a	3.66ba	2.64a	4.78a	4.09a	5.32a	23.54a

Means with the same letters in the same column are not significantly different at 0.1% level of significance ( $p=0.0001$ ).

The LC<sub>50</sub> values of the bio-active ingredients of Neem and Papaya were determined against three developmental stages of *B. tabaci* under controlled laboratory conditions and using a stock solution of 1mgmL<sup>-1</sup> serially diluted into six concentrations and results are presented in Table ( 4). The LC<sub>50</sub> values for 2<sup>nd</sup>, 3<sup>rd</sup> instars and Pupa treated with Neem extract were 8.09, 10.67 and 15.25 respectively. A similar trend was observed when the *B. tabaci* immatures (2<sup>nd</sup> instars, 3<sup>rd</sup> instars and Pupa) were treated with Papaya extracts indicating LC<sub>50</sub> values of 10.37, 12.25 and 18.47 respectively (Table 4).

The 2<sup>nd</sup> instars for both treatments were more susceptible than the and required least concentration to inflict 50% mortality. The order of susceptibility for neem and papaya were 2<sup>nd</sup> instars > 3<sup>rd</sup> instars > Pupa (Table 4). The probit analysis correlation values showed strong relationship between the concentrations of the active ingredients and the developmental stage with correlation coefficient ( $r^2$ ) values > 0.75 target population that were tested (Table 4)

**Table 4:** LC<sub>50</sub> values of Neem and Papaya extract against *B. tabaci* immatures

Bio-active ingredients components	<i>B. tabaci</i> Developmental stages	LC <sub>50</sub> values (mg/mL)	R <sup>2</sup>
Neem	2 <sup>nd</sup> Instars	8.09	0.89
	3 <sup>rd</sup> Instars	10.67	0.81
	Pupa	15.25	0.80
Papaya	2 <sup>nd</sup> Instars	10.37	0.79
	3 <sup>rd</sup> Instars	12.25	0.85
	Pupa	18.47	0.75

Lethal concentration (LC<sub>50</sub>) of Papaya extracts required to effect 50% mortality of *B. tabaci* immatures. Each batch (n= 30 ) immatures assayed at various concentrations of Papaya extract i.e. active ingredients for an incubation period of 8 days.

Results of LT<sub>50</sub> values of neem and papaya extracts against *B. tabaci* immatures maintained under laboratory conditions are presented in (Table 5). The LT<sub>50</sub> values varied with development stages with a trend 2<sup>nd</sup> < 3<sup>rd</sup> instars < Pupa . The LT<sub>50</sub> values for population treated with neem extract are slightly reduced than population treated with papaya extract showing a variation in toxicity. Results showed a strong correlation coefficient indicating that killing of individuals in the population are time and stage dependent with 2<sup>nd</sup> instars requiring shortest period of time 2.54- and 4.63 days for populations treated with neem and papaya to inflict 50% mortality while the pupa required more time to kill 50% 7.11 and 11.7 days of the individuals for both the Neem and Papaya extracts respectively . It is however noted that populations treated with neem indicated relatively shorter periods for the three developmental stages as compared to population treated with Papaya extract (Table 5).

**Table 5:** LT<sub>50</sub> of Papaya and Neem extract assayed against *B. tabaci* Immatures

Bio-active ingredients components	<i>B. tabaci</i> Developmental stages	LT <sub>50</sub> values (days)	R <sup>2</sup>
Neem	2 <sup>nd</sup> Instars	2.54	0.92
	3 <sup>rd</sup> Instars	6.23	0.79
	Pupa	7.11	0.88
Papaya	2 <sup>nd</sup> Instars	4.63	0.84
	3 <sup>rd</sup> Instars	8.66	0.83
	Pupa	11 .47	0.75

Lethal Time (LT<sub>50</sub>) of Papaya and Neem extracts required to effect 50% mortality of *B. tabaci* immatures . Each batch (n= 30 ) immatures assayed at a concentration 1mgmL<sup>-1</sup> of Papaya and Neem extract a cumulative mortality computed for 12 days.

The impact of neem and papaya extracts on demographic parameters of *B. tabaci* are presented in (Table 6). The Gross Reproductive rates (Ro) varied, with the highest reproductive rate 23.56 eggs observed on control population whilst the least was noted on

population treated with Papaya extract at  $1\text{mgmL}^{-1}$  whilst no significance difference was observed for population treated with neem extract and control ( $p=0.0001$ ) (Table 6). The intrinsic growth rates ( $r^m$ ) on insect population treated with papaya, neem extracts and control were 0.101, 0.098 and 0.123 respectively. The Generation Time (GT) were fairly constant irrespective of treatment (Table 6). The Double Time (DT) were computed as 6.860, 7.073 and 5.635 for populations reared on papaya, neem extracts and control respectively.

**Table 6:** Comparison of the Neem and Papaya Concentrations  $1\text{mgmL}^{-1}$  on Demographic Parameters of *B. tabaci* reared on poinsettia plants and maintained under standard laboratory conditions  $25\pm 2^\circ\text{C}$ .

Treatments	Ro	Td	$r^m$	$\lambda$	GT	DT
Papaya Extract	18.97a	29.01	0.101	1.106	29.137a	6.860a
Neem Extract	13.66 b	26.58	0.098	1.103	26. 670a	7.073a
Tween 80	23.56 a	25.63	0.123	1.130	25.681a	5.635a

## DISCUSSION

*B. tabaci* is one of the most serious economic pests affecting a wide range of crops including vegetables, ornamental and fruit tree crops debilitating the production, productivity and economic values of these crops. The management of this pest has been prioritized due to its economic status, and has been significantly controlled by the use of broad-spectrum chemical insecticides. Despite the ability of the pesticides to reduce the pest population below the economic injury level or action threshold, the side effects normally over shadow the essence of their use, hence alternative measures of pest management besides chemical pesticides is always sought. More recently the resurgence of interest on the use of botanical bioactive ingredients has emerged as a recent field in pest management as the inherent values are being underscored as a promising alternative strategy to chemical pesticides as this category of biopesticides do not cause any devastating environmental degradation or injury to non-target beneficiary organisms like natural enemies and pollinating agents. Additionally, [19] [20] opined that plant-derived products are best suited for use in organic food production and in the production and postharvest protection of food in developing countries. In this context, the Integrated Pest Management Unit, Department of Horticulture, Njala University is undertaking an ongoing research efforts to evaluate insecticidal significant of native plants derivatives as a strategic pathway in the management of agricultural pests. Among the numerous available plants which can be easily and locally sourced, Neem and Arica papaya are quite common and found growing in diverse ecological zones exhibiting a potential of demonstrating broad-spectrum activities against a wide range of horticultural pests including whiteflies [27] [28] [29] based on the chemical compositions supported by ample literature documentation. Their potency as biopesticides is often displayed through multiple mechanisms such as antifeedant, oviposition deterrent, growth inhibition disrupting the physiological and hormonal activity of insects thereby reducing the tendency of increasing in number. This mode of action exemplifies these botanical derivatives as suitable sustainable alternative to broad-spectrum synthetic

chemical pesticides that can be exploited as effective substitutes to broad spectrum synthetic chemical pesticides [27] [28].

The impact of bioactive ingredients of neem and papaya assayed against eggs incubated under laboratory conditions did not inhibit egg hatchability, as over 70% of the treated eggs hatched without hindrance with no significant difference between the control and the treated eggs ( $p > 0.0001$ ). Study carried out by [27] [28], however observed that aqueous solution of Neem extract at conc of 8mg/mL inhibited egg hatchability more effectively reducing hatch rate to as low as 20-30% under laboratory conditions. Comparatively, the results of [27] support the findings in the current study due to difference in concentration of the active ingredients used in the studies. Despite the low rate of egg hatchability, the authors argued that that not all neem formulations affect egg hatchability as demonstrated in the case where neem seed oil encapsulated  $\beta$ -cyclodextrin showed no significant impact on egg viability in soybean greenhouse assay. In another research efforts, [25] clarified that aqueous solutions of neem primarily target larva stages than eggs. This view is evidenced in the result obtained in this investigation where % larval mortality was quite significant ( $p < 0.0001$ ) with high % larval mortality recorded in the 2<sup>nd</sup> and 3<sup>rd</sup> instars. The bioassay of papaya extract on the % egg hatchability showed similar results as that of Neem, there was significant % hatchability, but cumulative larval mortality was significantly high for the immatures indicating that aqueous extracts exhibit high toxicity against the immatures. The physiological disruptive behaviour of both Azadirachtin of neem and papain of papaya interfering cellular processes has been evidence as a mortality factor in immatures of sap sucking insects like *B. tabaci*. Studies carried out [26] [27] noted that no specific ovicidal effects of aqueous extract of papaya but opined that there is high mortality in nymphal and adults stages of *B. tabaci* population reared on chilli pepper reducing population about 70% at high concentrations. The extracts of *A. papaya* and Neem were bio-assayed against a batch of *B. tabaci* eggs on poinsettia plants to determine egg hatchability which is a unique feature that determines the propensity or initial phase of population explosion. It was observed that agrochemicals including bio-pesticides have shown sub-lethal effects on egg hatchability disrupting the morphological, hormonal and physiological behavioral according to [26]. Despite these unique features, [27][24] opined that the potency of botanical derivatives against targeted developmental stages is highly dependent on the method of extraction and formulation, and concentrations of the plant derived products. The current investigation employed ethanol extraction for *A. indica* and *A. papaya* on effects on egg hatchability. Results indicated no depressive effect on % hatchability, as greater percentage of hatchability was observed with over 70% of the eggs hatched to crawlers. A study carried out by [26], investigating the sub-lethal effect of Neem extracts on the hatchability of *B. taabci* eggs reared on collard host plant indicated about 30% reduction in hatchability as compared to untreated control. The findings of these authors are similar to current findings where about 35% of eggs failed to hatch when treated with 1mg/mL concentrating of neem extract and papaya extracts respectively. A similar study by [28] [29] sub-lethal effects of ethanolic and methanolic extracts from Neem accounted for 13% and 17% reduction egg hatchability at 1.5g/L over 15 days. [25] studied the impact of sub-lethal effects of neem extracts against *B. tabaci* under field conditions opined that Neem extracts, rich in bioactive compounds like azadirachtin, exhibit sub-lethal effects on pest reproduction, including reduced egg hatchability, often through disruption of hormonal balances such as ecdysteroid and juvenile hormone titers, leading to impaired oogenesis and decreased viability of eggs without immediate lethality at lower doses. The authors

emphasized that the effects are concentration-dependent and manifest as partial inhibition of hatching, morphological deformities in embryos, or delayed development, contributing to overall population suppression in integrated pest management. The results derived from this study followed do not strictly follow the reason of [29] [30] where hatchability was dose or concentration dependent as the highest concentration  $1\text{mg mL}^{-1}$  did not show significant suppressive effect on hatchability of *B. tabaci* eggs as compared to the lowest concentration  $0.2\text{mg mL}^{-1}$ , no significant effect was observed ( $p > 0.0001$ ).

With respect to *A. papaya* extract at different concentrations tested against the *B. tabaci* eggs and larval mortality, results indicated that the sublethal effects of extract solution showed minimal effect on hatchability at all the observed concentrations as results did not exhibit any significance differences ( $p > 0.0001$ ), however larval mortality was dose-dependents with significant mortality ( $p < 0.0001$ ) of the immature in the order  $2^{\text{nd}}$  instars  $> 3^{\text{rd}}$  instars  $>$  pupa. [30] reported that *A. papaya* leaves contain phytochemical steroids, saponins, triterpenoids, lipids, coumarins and other organic lipids showing a high potential biopesticide features which can be exploited for pest management. Ishak et al (2019) showed that papaya leaf extracts showed a significant effect on larval mortality when treated on anopheles' mosquito larval indicating about 30% mortality of larvae, percentage of mortality was significantly increased when the leaf extract was combined with papaya seed extracts on the hatchability of cowpea bruchids with over 50% mortality at higher concentrations. In this context, plant-derived products are best suited for use in organic food production and in the production and postharvest protection of food in developing countries [18] [19].

$LT_{50}$  and  $LC_{50}$  are parametric indices used to determine the kinetics and toxicity of pesticides, plants bio-active ingredients or bio-pesticides against sap sucking insects specifically that feed on phloem saps. These parameters are quantitatively used to quantify time and concentration required to inflict 50% mortality in insect population. The current study indicated that  $2^{\text{nd}}$  and  $3^{\text{rd}}$  instars were more susceptible to extracts indicating low  $LC_{50}$  and  $LT_{50}$  values as compared to the pupa stage. The  $LC_{50}$  values for  $2^{\text{nd}}$ ,  $3^{\text{rd}}$  instars and Pupa treated with Neem extracts were 8.09, 10.67 and 15.25 respectively whilst batches of immatures treated with *A. papaya* extracts indicated  $LC_{50}$  of 10.37, 12.25 and  $18.47\text{ mg mL}^{-1}$  respectively. Though the immatures showed over 60 % cumulative mortalities observed when the immature populations were treated with the extracts the  $LC_{50}$  and  $LT_{50}$  values however varied for each specific stage with  $LC_{50}$  and  $LT_{50}$  values showing a trend in the order  $2^{\text{nd}}$  instar  $< 3^{\text{rd}}$  instars  $<$  Pupa. The pupa population treated with Neem and Papaya extracts required 7.11 and 11.47 days and 15.25 and  $18.47\text{ mg mL}^{-1}$  respectively to achieved 50% mortality ( $p > 0.0001$ ). This invariably indicates that more time and higher concentrations were required in the case of papaya treated population. The potency of *A. indica* ethanolic extracts exceeds that of *A. papaya* in the current study. [24] [18] studied the insecticidal effects of plant extracts such as *Azadirachta indica* and other native plants on immature whitefly *B. tabaci*, results indicated that most of the aqueous and ethanolic extracts showed high insecticidal effects on *B. tabaci* eggs. The lowest  $LC_{50}$  values were recorded in the aqueous extracts of tested plants. On the other hand, *B. tabaci* nymphs were not affected by the aqueous extracts, but were highly sensitive to the ethanolic extracts of the tested plants. The lowest  $LC_{50}$  values were recorded in the ethanolic extracts of *P. alliaceae* ( $1.27\text{ mg mL}^{-1}$ ) and *T. arborea* ( $1.61\text{ mg mL}^{-1}$ ).

Their studies clearly indicated that the insecticidal effectiveness of plant derived botanicals on *B. tabaci* immature depend on the nature of extractions formulation and concentration of plant derivatives on sucking insects like *B. tabaci* . The potency of pesticides including plant derivatives are measured in terms of the kinetics i.e. rate or speed of infection and mode of action as inscribed in the  $LT_{50}$  and  $LC_{50}$  . Besides these quantitative arbitrations, the developmental stage is key and is observed to influence the outcome of these values as indicated in the current studies. Though [24] [18] have underscored the ovicidal potency of both neem and papaya extracts, however the % hatchability data derived from the study differed from the views of these authors as a significant percentage > 60 eggs hatched into 1<sup>st</sup> instars (crawlers) , but larval mortality was significantly high ( $p < 0.0001$ ).

Demographical parameters are valuable tools used to determine the nature and progression of population growth. These parameters can be described in terms of finite growth ( $\lambda$ ) , the intrinsic growth ( $r^m$ ) , the gross reproductive rates ( $R_0$ ) , the generation time (T) and double time (DT) which provide valuable insight on population trend [34][37]. However, these parameters can be influenced by intrinsic and extrinsic factors either by promoting or suppressing population growth [35]. The demographic results strongly revealed that cohort of eggs treated with the extracts incubated and monitored to adult emergence, the intrinsic growth rates  $r^m$  which is a summarized growth parameter influenced by intrinsic and extrinsic were computed as 0.101, 0.098 and 0.123 for populations treated with papaya, neem extracts and Tween 80 respectively. The  $r^m$  value of population treated with neem extracts was depressed to 0.098 as compared to the population challenged by the papaya extract and the control. The  $r^m$  value of the population treated with neem extracts influenced the fertility, gross reproductive rate  $R_0$  thereby extending the finite growth rate  $\lambda$  and overall population growth which can be measured in terms of generation time i.e. from oviposition to adult emergence. These results shared parallel views of [27] who underscored that aqueous extracts of some medicinal plants are as toxic as imidacloprid to sweet potato whitefly disrupting the physiological and hormonal functions that potentially deter the normal growth and development of insects. The extracts of *A. papaya* and *A. indica* showed toxicity in reducing the reproductive potential of *B. tabaci* in the current studies when the gross reproductive capacity ( $R_0$ ) of population treated with extracts were compared to the control.

The ongoing research in investigating plant extracts is a preliminary effort to provide a baseline information on the potential of plant derivatives as biopesticides for management of agricultural pests. The potential of bio-pesticides as substitutes to broad-spectrum chemical pesticides a laudable effort and this initiative of using biopesticides will be quite feasible and a kind of sustainable practice according to [19] meeting the requirement of using natural products underlining organic farming within the context of Sierra Leone.

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## Authors' Contribution

The authors significantly contributed to the realization of this research.

## Conflicts of Interest

No conflict of interest in field, laboratory and data analysis. The authors declare no conflicts of interest regarding the publication of this paper.

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