THESIS

THE BIOLOGY OF Pentalonia nigronervosa LIVED ON RODENT TUBER (Typhonium flagelliforme) AND ITS EFFICIENCY AS BANANA BUNCHY TOP VIRUS VECTOR

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PLANT PROTECTION STUDY PROGRAM
FACULTY OF AGRRICULTURE
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This thesis was written to fulfill one of the requirements to accomplish Bachelor Degree (S1) of Plant Protection at Faculty of Agriculture, Sriwijaya University

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SUMMARY

Yunanda Audri Balqis, *The Biology Of Pentalonia Nigronervosa Lived on Rodent Tuber (Typhonium Flagelliforme) And Its Efficiency As Banana Bunchy Top Virus Vector* (Supervised By **Bambang Gunawan**).

P. nigronervosa is the main vector for Banana Bunchy Top Virus (BBTV) the causal agents of Banana Bunchy Top Disease (BBTD), which causes the infected bananas have bunchy top appearance and bear no fruit. This research consisted of two experiments. The first was bioassay of *P. nigronervosa* lived on rodent tuber. Morphology and biology of *P. nigronervosa* lived on rodent tuber were observed and measured on 10 young sucker of rodent tuber in laboratory of Entomology. The second was an experiment on the effect of rodent tuber on the efficiency of BBTV transmission by *P.* nigronervosa. The experiment was arranged in a randomized Blok Design with 4 level of treatment and 10 replications. The treatment was time period of passing viruliferous vector on rodent tuber consisted of 4 levels i.e., 0, 24, 48 and 72 hours. Each treatment was replicated 10 times and each treatment unit consisted of 3 banana plants. The results showed that *P. nigronervosa* could live and breed on rodent tuber with morphology and biology not different from those lived on banana. The aphid had 4 instars of nymph and one phase of imago. The imago was wingless, blackish brown in color with a body length ranging from 1.4 mm to 1.7 mm. Rodent tuber did not affect the efficiency of BBTV transmission by *P. nigronervosa.*

Keywords: *P. nigronervosa*, BBTV, Banana, Rodent tuber

APPROVAL SHEET

THE BIOLOGY OF Pentalonia nigronervosa LIVED ON RODENT TUBER (Typhonium flagelliforme) AND ITS EFFICIENCY AS BANANA BUNCHY TOP VIRUS VECTOR

THESIS

As one of one of the requirements to accomplish Bachelor Degree (S1) of Plant Protection at Faculty of Agriculture, Sriwijaya University

By:

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Indralaya, December 2021

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Thesis entitled "*The biologi of Pentalonia nigronervosa lived on rodent tuber and its efficiency as banana bunchy top virus vector" by* Yunanda Audri Balqis had been defended in fornt of Thesis Examination Committee of Faculty of Agriculture, University of Sriwijaya on 21 December 2022 and had been revised accordingly as suggested by examination board team members.

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Hereby stated that all of data and information presented in this thesis is my own result of observation under my supervisor, except those clearly mentioned their source. If in this thesis is found any plagiarism in the future, I will be ready to accept academic punishment from University of Sriwijaya.

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Indralaya, December 2021
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BIOGRAPHY

The author was born in Sidorejo, Pagaralam City on 25 June 2000, the first daughter of Mr. Wanhar Husein and Mrs. Eva Hartini. The author completed her Elementary School in SDN 2 Muara Payang, Lahat, Yunir High School in MTS Raudhatul Ulum Sakatiga Indralaya, and Senior High School in SMA Muhammadiyah Pagar Alam. The author completed her senior high school in 2018 and continued her study to Department of Plant Protection, Faculty of Agriculture, Sriwijaya University via National Selection for State University.

During her enrolment as student in Sriwijaya University, the author took part as a member of Besemah Pagaralam Student Association and Plant Protection Student Organization. In 2021 the author was assigned as practicum assistant of Vertebrate Pest Course.

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I would like to extend special thanks to The Mightiest Allah SWT for the blessing and grace for me, so that I can finish research and write this thesis on time. I also want to apologize for inappropriate words I might unconsciously use in this thesis. I do realize that the research behind this thesis could be completed properly only because of the support from various parties. I have to thank to my parent for their invaluable supports, loves and invocation. Thanks to Madam Nurli Hasanah who has taken care of me during her study, and to my younger brother Dzaki Fadlur Rahman for his support and fun.

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Finally, I want to thanks to all Plant Protection students, especially the class of 2018 for all the joy and melancholy we had share together in Indralaya.

Indralaya, December 2019

The Author

CONTENTS

CHAPTER 1
INTRODUCTION

1.1 . Background

One of promising fruit crops in Indonesia is banana. Extensification of banana cultivation is aimed at fulfilling the needs of increasing fruit consumption along with the increase of people's knowledge on nutrition values, because banana contains minerals, vitamins and carbohydrate. Besides having good taste, nutritious and relatively affordable, banana is economically important and promising because almost all people in all countries like the fruit (Kasrina and Zulaikha, 2013). Banana production in 2020 was 8 182 756,00 tonnes (Badan Pusat Statistik, 2020). During the last five years, banana production was increasing. However, banana cultivation would not face technical obstacles or regulation. Pest and diseases have been the important limiting factor for banana production. Several diseases could reduce the quality as well as quantity of banana product. *Fusarium oxysforum*, the causal agent of banana wilt disease, and Rastolnia *solanacearum*, the causal pathogen of bacterial disease has been reported as important diseases of banana. However, Banana Bunchy Top Disease caused by Banana Bunchy Top Virus is believed to be the most damaging disease of banana (Sariamanah, Munir and Agriansyah, 2016).

Banana bunchy top virus (BBTV) causes infected banana plant stunt and bunchy appearance. In Indonesia, the disease was firstly reported to be found in Cimahi and Padalarang in 1978. The disease incidence was around 21,52% to 55,23%, and now the disease has spread to menyerang perkebunan pisang di Java, Bali, Kalimantan, Jayapura, Lampung and South Sumatera (Widyastuti and Hendrastuti Hidayat, 2005). Symptoms appear on banana plant infected by BTV are the formation of dark green broken line along leaf veins, leaf narrowing with chlorotic on the leaf margins, and fragile (Irwansyah, Sofian and Akhsan, 2019). The infected plant fails to produce fruit and if the plant infected at later stage of its development, the plant still produce fruit but the fruit is abnormal and not marketable (Watanabe and Bressan, 2013).

Banana Bunchy Top Virus (BBTV) is spread by *P. nigronervosa*. *P. nigronervosa* in persistent manner, the virus was circulated in the vector's body and the vector is able to transmit the virus continuously. Instead of infesting banana, *P. nigronervosa* was also found to infest other plant species such as ginger, galangal, taro and cardamom (Suparman, Nurhayati and Setyawaty, 2011). Banana aphid *P. nigronervosa* has four nymph instars. The newly born first instar nymph is pear-shaped, reddish in color with four segment antennae at 0.12 mm length on average. Second instar is similar to first instar with body length 0,8 mm. Third instar is brown in colour with body length 0,9 mm. In this phase, the eyes are obvious and the antennae have 5 segments. Fourth instar has had 6 segment antennae, light brown in colour, and the body length has reached 1,2 mm.

P. nigronervosa transmit and spread stunt disease of banana caused by Banana Bunchy Top Virus. The virus is circulated in the vector body but no replication of the virus particles occurs and no transovarial virus transmission. The virus remains in the parent body when the viruliferous aphid gives birth to its progeny (Suparman, *et al*, 2017). In order to be infective vector, *P. nigronervosa* has to find infected banana plant and needs at least 4 hours acquisition feeding on the plant. However, most vector gain enough number of virus particles after passing acquisition feeding for 18 hours. After the vector being infective, the vector can retain the virus in its body for 15 - 20 days, or even for the rest of its life time (Kurnianingsih, Ghazali and Astuti, 2018).

Rodent tuber (*Typhonium flagelliforme*), is a member of Family Araceae, which recently draws more attention because of its potency for curing cancer, even though there is still a need to further study of its potency as medicinal plant (Sianipar, Purnamaningsih and Rosiana, 2016). The name given to the plant is believed to be related to the one organ of the plant. Flower appendix of the plant is resemble rodent tail, while other parts of the plant are similar to those of other Araceous plants, but rodent tuber is shorter than other plants in the family. (SYAHID, 2020). Rodent tuber plant is only 10-20 cm tall and grows optimally under shaded and humid areas (Rachman, 2012).

1.5. Problem Formulation

Based on the background previously mentioned, there were two research problems to solved, as follow:

1. How did rodent tuber affect the morphology and biology of *Pentalonia nigronervosa* lived on the plant?
2. How did rodent tuber affect the efficiency of BBTV transmission by *Pentalonia nigronervosa* when infective *P. nigronervosa* stay for different period on rodent tuber before feeding on healthy banana?

1.6. Research Objectives

The objectives of the research were as follow:

1. To record morphology and biology of *Pentalonia nigronervosa* lived on rodent tuber (*Typhonium flagelliforme)*
2. To find out the effect of rodent tuber (*Typhonium flagelliforme*) on the efficiency of BBTV transmission by Pentalonia *nigronervosa.*

1.7. Research Hypothesis

The hypotheses of the research were as follow:

1. *Pentalonia nigronervosa* would be able to live and breed on rodent tuber with morphological and biological characteristics different from the aphid lived on banana.
2. Rodent tuber would be able to reduce the efficiency of BBTV transmission by *P. nigronervosa* after the viruliferous aphid stay on the plant for different period before feeding on healthy banana.

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1.8. Research Usage

The research was expected to provide information on the morphology and biology of *P. nigronervosa* when the aphid lived on rodent tuber as alternative host. Furthermore, it was also expected that the research would verify that rodent tuber could reduce the efficiency of BBTV transmission by *P. nigronervosa.*

CHAPTER 2
LITERATURE REVIEW

2.2. Banana Plant (*Musa* spp.)

Figure 2.1. Banana Plant (Sasmita Mokolintad, *et al.*2003)

Banana is a monocot plant, have only one trunk without any branch. The so-called stem of the plant is actually leaf sheaths tightly packed and overlapping to make a structure looks like a trunk. The 'true' stem provides support to the leaves and flowers and fruits. The leaves and flowers are attached to a node, and the sections between nodes are internodes. A lateral shoot that develops from the rhizome usually emerges close to the parent plant and is called as sucker (Arifki and Barliana, 2018). Banana has organs in common such as root, stem, leaf, flower, fruit and seed. Root of banana is fibrous root and the plant has no anchor root. The rhizome or *corm as the real stem* is characterized by horizontal underground growth. The corm is vertical enlarged compact stem with roots produced from every node. A lateral shoot develops into and usually emerges close to the parent plant. A sucker that has just emerged through the soil surface is called a *peeper*. A full-grown sucker bearing foliage leaves is called a *maiden* sucker.

Banana leaf emerges from the center of the pseudo stem as a rolled cigar leaf. The petiole becomes the midrib, which divides the leaf blade into two lamina halves and gives special appearance of banana leaf (Kasrina and Zulaikha, 2013).

2.1.1. Banana Taxonomy

According to (Anggoro, 2016), the classification of banana is as follow:

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Liliopsida
Order	: Zingiberales
Family	: Musaceae
Genus	: Musa
Species	: Musa acuminata

2.1.2. Banana Cultivation

Banana grows optimally in tropical areas, both in low and high altitude but not more than 1600 m above sea level. The optimum temperature for banana growth is 27°C, with maximum temperature of 38°C, and soil pH 4.5-7.5. Optimum rainfall 2000-2500 mm/year or a minimum of 100 mm/month. In an area with dray condition more than 3 consecutive months, banana plant needs watering for its optimum growth and reproduction. (Sariamanah, Munir and Agriansyah, 2016).

Most banana are cultivated in a mixed cropping pattern where banana is planted together with cassava, ginger, pineapple, turmeric, taro and various field crops which grow continuously along with banana and its suckers. Banana also frequently planted in an intercropping patter, where banana planting is followed by planting of other plant suitable to the land and climate condition. Some farmers use Colocasia, ginger, turmeric, galangal, maize and other annual crops as intercrop.

2.2 Banana Aphid (*Pentalonia nigronervosa*)

Figure 2.2. Banana Aphid *Pentalonia nigronervosa* (Sasmita Mokolintad, *et al*, 2003)

According to Muthia (2017), the classification of banana aphid is as follow:

Phylum : Arthropoda
Class : Insecta
Order : Hemiptera
Family : Aphididae
Genus : Pentalonia
Species : *Pentalonia nigronervosa* Coquerel

Banana aphid *Pentalonia nigronervosa* is widely distributed all over the world in both hemispheres, especially in tropical countries (Blackman and Eastop 2000). Even though *P. nigronervosa* is called as banana aphid, the aphid is not only able to live on banana plant, but also able to live on other planta species, especially Family Zingiberaceae such as cardamom (Elettaria), comb ginger (Alpinia), ginger (Zingiber), and Araceae such as taro (Colocasia), Caladium, Costus, Dieffenbachia, Hedychium, Heliconia and Xanthosoma (Suparman, *et al*, 2015).

2.2.1. Morphology and Biologiy of *Pentalonia nigronervosa*

Banana aphid *P. nigronervosa* is a parthenogenic insect. The insect reproduces without fertilization and the population purely consist of females. Life cycle of the aphid, from newly born nymph to the delivery of new progeny ranges from 9-16 days and the imago life span ranges from 8 toi 60 days. The imago begins to reproduce 1 day after the last molting, from fourth nymph instar to imago. One adult female produces 14 progenies on average. Banana aphid *P. nigronervosa* passes 4 instars of nymph before molting to adult imago. The first instar is pear-shaped, reddish brown in color, and has 4 segment antennae at 0.1 mm length. Second instar is similar to the first instar, but with body length Nimfa 0,8 mm. Third instar is brown in color with body length 0,9 mm. At this instar, eyes and antennae are obvious with antennae consists of 5 segments. Fourth instar is light brown in color with body length 1 mm and has 6 segment antennae (Rahmah, Maryana and Hidayat, 2021).

P. nigronervosa is not a good flyer and because of its small size, the aphid can be brought by wind to long distance. Its flying activities are between 09.00 to 11.00 AM and 5 PM. Therefore, BBTV can be transmitted from infected plant to surrounding banana plants and also can be transmitted to banana in long distance areas. The aphid is commonly found in a colony under young leaf petiole or around leaf midrib. The aphid lives on the lower part of the plant host frequently make association with ant. The ant feed on honey dew secreted by the aphid and the aphid are protected by ant from natural enemies, especially predators. As a vector of BBTV, *P. nigronervosa* has to feed on infected banana plant for at least 4 hours to infective, but mostly need acquisition period up to 18 hours to take enough titer of the virus particles. After being infective, the vector can retain and able to transmit the virus for 15 to 20 days or during the rest of the vector’s life (Dina, 2018).

2.3 Banana Bunchy Top Disease

Figure 2.3. Symptom of Banana Bunchy Top Dizease (Sutrawati and Ginting, 2009)

Banana Bunchy Top Disease (BBTD) is caused by Banana Bunchy Top Virus (BBTV) which is a member of Genus Babuvirus, Nanoviridae. BBTV is transmitted by *P. nigronervosa* Coquerel and also spread to long distance trough sucker transportation when the sucker infected by the virus but with no clear symptomn (Keiko T. Natsuaki. *et al.* 2007). The disease is economically important in Indonesia. According to previous reports, the disease was firstly found in Java and Bali, but soon the disease has been found in 7 provinces in Indonesia i.e. Lampung, Riau, West Sumatra, West Java, Central Java, Yogyakarta and Bali (Hidayat, 2019).

BBTD is transmitted persistently by *P. nigronervosa*¸ the aphid frequently stay at the lower part of the plant, near the ground surface and prefer to infest young newly emerging suckers (Robson and Wright, 2007). The aphid pierces the host plant to the phloem and sucks phloem liquid. The sucking activity is used by the virus to follow the liquid flow through the stylet and then circulated inside the aphid body, which eventually reaches the saliva gland. The virus particles will be transmitted to new plant host when the aphid suck healthy plant and lubricate the stylet using saliva. The early symptom of BBTV is quite difficult to detect but as the disease develops, the symptoms on the plant leaves getting more obvious. The leaves are narrower and erect and group together to make bunchy appearance. The leaves getting fragile and chlorotic as well as necrotic appear on the leave margins. (Irwansyah, Sofian and Akhsan, 2019).

2.5. Rodent tuber

Figure 2.4. Rodent tuber plant (Syahid, 2020)

Rodent Tuber (*Typhonium flagelliforme Lodd*.) is an herbal plant belongs to Family Araceae. The plant is distributed in several tropical countries such as India, Indonesia Malaysia Sri Lanka and Australia. Rodent tuber can grow optimally in low land and high land with optimal altitude ranges from 1 to 300 m above sea level and more suitable to shady and humid areas (Sianipar, *et al*, 2016). Rodent tuber is relatively smaller compared to other species of Araceae, only 10 to 20 cm high. The plant is so called as rodent tuber because its flower has an appendix that resemble rodent's tail. The plant can only be reproduced vegetatively by using its sucker.

Rodent tuber contains medicinal compounds such as alkaloids, flavonoids terpenoids, steroids, antioxidants and antibacterial compounds. The plant has also been reported to be used as anticancer. The plant is reported to be able to kill cancer cells and this has made the plant more interesting as medicinal herb (Hesananda et al, 2017).

2.4.1. Classification of Rodent Tuber

According to (Hesananda, *et al*, (2017), the classification of rodent tuber is as follow:.

Phylum : Traheophyta
Class : Liliopsida
Order : Arales
Family : Araceae
Genus : *Typhonium*
Species : *Typhonium flagelliforme* (Lodd.) Blume

2.4.2. Morfologi Tanaman Keladi Tikus

Rodent tuber (*Typhonium flagelliforme*) is well known as Rodent Tuber due to an appendix of its flower resemble rodent tail. Rodent tuber is an herbal plant, relatively small measuring 10 t0 20 cm in height, even though in certain condition the plant may reach 40 cm high. Young leaves are oval in shape and the mature ones look like triangle, rounded at the bottom and narrowing to the tip. Leaves are green in color, the petiole also green in color but getting pale and whitish to the basal of the petiole. Leaf surface is flat and smooth without trichome. The rhizome is brown with black dots on the surface and white inside, thin skin and easy to peal (Syahid, 2008).

2.4.3. Habitate and Distribution

Rodent tuber grows in tropical and sub-tropical areas, from low to highland, up to 100 m above sea level. The plant grows optimally in shady and humid areas. Ini Indonesia, rodent tuber is found in most areas of Java, Sumatra, Kalimantan and Papua (Rachman, 2012).

CHAPTER 3
RESEARCH METHODOLOGY

3.1. Time and Place

The research was conducted in Insectarium, laboratory of Entomology, Department of Plant Pest and Disease, Faculty of agriculture, Sriwijaya University from July to December 2021.

3.2. Equipment and Materials

Equipment used in this research included: 1) stationary, 2) bucket 3) chopper 4) hoel, 5) sprayer, 5) small and big plastic cups, 6) cutter, 7) scissors, 8) micro lens, 9) refrigerator, 10 binocular microscope.

Materials used in the research included 1) banana suckers. 2) *Pentalonia nigronervosa*, 3) dung compost 4) polybag. 5) cheese cloth, 6) rodent tuber, 7) mineral soil, 8) cotton, 9) water

3.3. Methodology

The research consisted of two experiments; the first experiment was bioassay of *P. nigronervosa* live on rodent tuber. We use 10 rodent tuber suckers to investigate and record the morphological and biological characteristics of *P. nigronervosa*.

Second experiment was a field experiment to investigate and analyse the effect of rident tuber on the efficiency of BBTV transmission by *P. nigronervosa*. Experiment was arranged in a completely randomized design with 4 treatment levels and 5 replications. The treatment was period of infective *P. nigronervosa* stayed on rodent tuber before being transferred to healthy banana for virus transmission, consisted of 4 levels i.e. 0, 24, 48 and 72 hours. Each treatment unit consisted of 3 banana plants, resulted in the need of 60 banana suckers.

3.4. Research Procedure

This research consisted of two steps of experiment, the first was to observe morphology and biology of *P. nigronervosa* lived on rodent tuber. The second was investigate the effect of rodent tuber *P. nigronervosa* on the efficiency of BBTV transmission by *P. nigronervosa.*

3.4.1. Rearing of *Pentalonia nigronervosa*

Figure 3.1. Rearing of *P. nigronervosa* on rodent tuber

Banana aphid *P. nigronervosa* derived from infected banana was transferred to healthy banana for multiplication. The rearing was co ducted in a room to protect the aphid from natural enemies and observation was conducted daily until the number of aphid progenies was enough to conduct bioassay in the laboratory and field experiment.

3.4.2. Bioassay of *Pentalonia nigronervosa* lived on Rodent Tuber

Rodent tuber was planted in polybags to produce young suckers enough to conduct bio assay in the laboratory, for which 10 young suckers were required. Ten suckers were harvested to be planted individually in a clear plastic cup with moistened cotton at the base of the cup. A newly born aphid progeny were transfer to each rodent tuber sucker inside the cup, and the cup was then individually enclosed with cheese cloth to protect the aphid from natural enemies. Observations were made continuously to record the change of morphology and biology of each aphid, and if the aphid died for whatever reason, the dead aphid was replaced with the newly born one and the observation was repeated from the beginning.

Parameters observed include number of instars; shape, size and color of each instar, life cycle, imago life span, reproductive period, and fecundity.

Figure 3.2. Ten plastic cups enclosed with cheese cloth with rodent tuber inside infested with single *Pentalonia nigronervosa* nymph for bioassay

3.4.3. Experiment on the Effect of Rodent tuber on the Efficiency of BBTV transmission by P. *nigronervosa*

Rodent tuber was expected to be able to reduce the efficiency of BBTV transmission by *P. nigronervosa* when viruliferous aphis were transferred from infected banana to rodent tuber to spend certain period before being transfer to healthy banana to transmit the BBTV virus. The aphid was given time to feed on infected banana for 48 hours before being transfer to rodent tuber to stay there for 0, 24, 48 and 72 hours. After spending time accordingly to the treatment, the aphids were the transferred to healthy banana to transmit the virus contained in their body. Observation was made to record the incubation period and calculate the disease incidence, as the result of BBTV transmission by the vector.

3.4.4. Land Preparation

Land used for experiment was cleaned from weed using grass cutting machine and let the cut grass dry on site. The growing weeds were then sprayed using herbicide until the land freed from weeds and ready to be processed using hoe.

3.4.5. Banana Planting

Banana suckers was grown from banana corm in a seedling bad. At age ± 2 months the suckers were transplanted to the prepared land. The suckers were planted in groups of three, each group for one replication of each treatment level, so we had 20 groups of three suckers with planting space 1.5 m between groups. Dung compost was applied before transplanting.

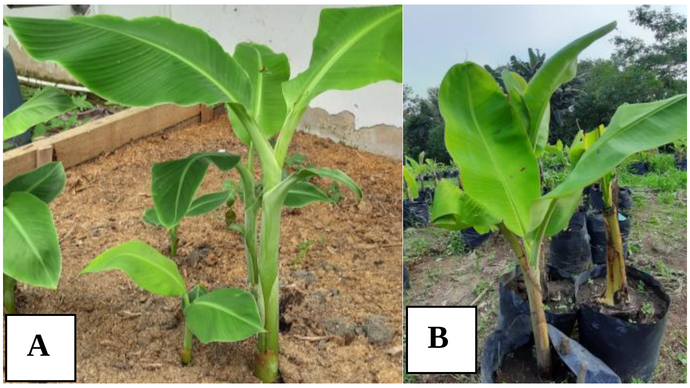


Figure 3.3. (A) Banana suckers grown in seedling bed, (B) Banana sucker ready for transplanting

3.4.6. Plant Watering

Plant watering was conducted every evening except on raining days using ground water derived from artesian well. Since banana is more tolerant to dry condition compared to other crops, watering did not need much water but only to make the soil moist.

3.4.7. Weeding

Weeding was conducted every time the weed grew around the experimental plots. Weeding was done manually, removing the weeds by hands. However, weeding was done using hoe or manual weeder for intense weeds.

3.4.8. Infestation of *Pentalonia nigronervosa*

Figure 3.4. Transfering *Pentalonia nigronervosa* to young sucker in the clear plastic cup

This experiment used the third instar of *P. nigronervosa* nymphs. The aphids were brownish in colour with 5 segment antennae and black colour had appeared on their tibia. The aphids were given acquisition period of 48 hours on banana infected by BBTV. After 48 hours, the aphids were moved to rodent tuber to stay there for a period according to treatment level i.e., 0, 24, 48 and 72 hours.

3.4.9. Transferring *Pentalonia nigronervosa* from Rodent Tuber to Healthy Banana

Gambar 3.5. *Pentalonia nigronervosa* yang dipindahkan ke pisang sakit

This experiment used the third instar of *P. nigronervosa* nymphs. The aphids were brownish in colour with 5 segment antennae and black colour had appeared on their tibia. The aphids were given acquisition period of 48 hours on banana infected by BBTV. After 48 hours, the aphids were moved to rodent tuber to stay there for a period according to treatment level i.e., 0, 24, 48 and 72 hours.

3.4.11. Parameter observed

Parameter observed for field experiment was parameter that could be used to verify whether or not rodent tuber affected the efficiency of BBTV transmission by *P. nigronervosa.* Incubation period dan disease incidence could become indications of the reduction of transmission efficiency if the incubation was longer and the disease incidence was lower.

Incubation period was defined as a period between the transmission of BBTV by *P. nigronervosa* until the first day the inoculated banana plant showed first symptom of BBTV infection. Observation was made every day.

Disease incidence was defined as the proportion of inoculated plant showing disease symptom. Because each treatment unit consisted of three plants, the disease incidence ranged from 33.3 to 100%.

Disease intensity was calculated based on disease score (Brooks, 1999) and Irwansyah et al., (2019) as follow:

Score	symptom
0	Plant is healthy, no disease symptom
1	Plant showed little stunting, leaf began to narrow and chlorosis just started on leaf margins.
2	Plant showed moderate stunting, moderate yellow on leaf margins, leaf moderately narrowing.
3	Plant showed severe stunting, chlorotic and necrotic on leaf margins, severe leaf narrowing.

Disease intensity was calculated using formula according to Agrios, (1997) and Irwansy ah *et al*. (2019).

$$\textbf{Disease intensity} = \frac{\sum(ni \times v)}{Z \times N} \times 100\%$$

Notes:

ni = number of plants showed certain disease score

v = disease crore

Z = the highest score

N = Total number of plants observed

3.4.12. Data Analysis

For bioassay experiment, collected data was presented in appropriate tables and for field experiment, collected data was analysed using ANOVA for Completely Block Design with 4 levels of treatment and 5 replications.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Results

4.1.1. Morphological and Biological data of *Pentalonia nigronervosa* lived on rodent tuber

Banana aphid *P. nigronervosa* could live and breed on rodent tuber. The aphid passed 4 instars of nymph and 1 phase of imago. Reproduction of *P. nigronervosa* was fully parthenogenetic. Based on observation results, there were differences of characteristics among instars such as colour, body length, body width, and instar period.

4.1.1.1 First Instar

First instar of *P. nigronervosa* lived on rodent tuber was whitish brown in color, but at newly born, the aphid was transparent brown (Figure 4.1)

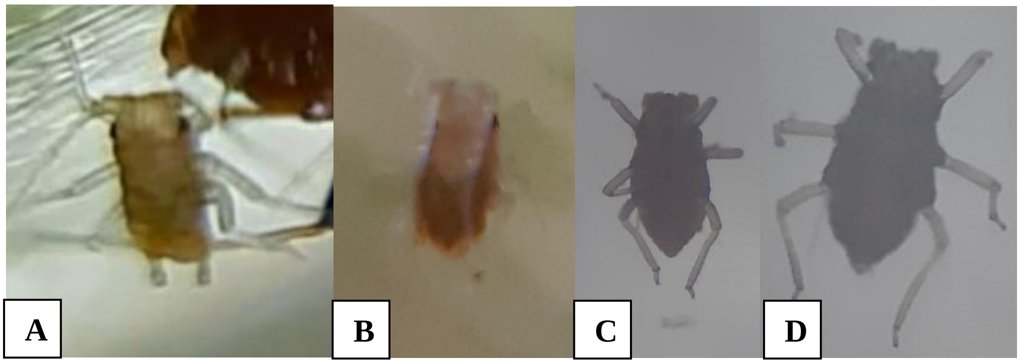


Figure 4.1. Banana aphid *Pentalonia nigronervosa* (A) newly born nymph, (B) one day old nymph, (C) first instar /, ventral, (D) first instar, dorsal.

Body length of first instar of *P. nigronervosa* raged from 0.61 to 0.69 mm and the width ranged from 0.28 to 0.37 mm (Table 4.1). The measurement was conducted one day after the aphid was born. The size of first instar could change along or getting bigger until first molting.

Table 4.1. Morphological characteristics of first instar nymph of *Pentalonia nigronervosa* lived on rodent tuber

Plant sample…	Morphological characteristic			
	color	Body length (mm)	Body width (mm)	Instar period (day)
1	Whitish brown	0.61	0.33	7
2	Whitish brown	0.61	0.29	6

3	Whitish brown	0.51	0.28	4
4	Whitish brown	0.63	0.34	7
5	Whitish brown	0.69	0.37	4
6	Whitish brown	0.66	0.33	3
7	Whitish brown	0.63	0.32	4
8	Whitish brown	0.76	0.33	4
9	Whitish brown	0.67	0.29	3
10	Transparent	0.67	0.32	3
Total		6.44	3.2	6.1
Average		0.644	0.32	0.61
Standard deviation		0.02	0.01	0.50

4.1.1.2 Second Instar

Second instar of *P. nigronervosa* nymph was pale brown in color with shape and size not too much different from those of first instar (Figure 4.2.)

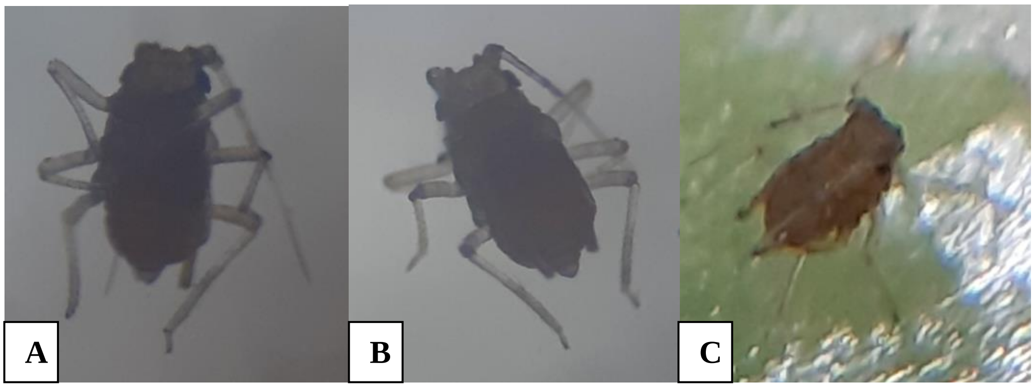


Figure 4.2. Banana aphid *Pentalonia nigronervosa* lived on rodent tuber (A) second nstar, ventral (B) second instar, dorsal, (C) color of second instar.

Body length of second instar of *P. nigronervosa* lived on rodent tuber raged from 0.71 to 0.83 mm and the width ranged from 0.36 to 58 mm (Table 4.2). The measuring activity was taken one after the nymphs molted from first instar to become second instar. The measurement of second instar size might be change since the period of second instar took 2-3 days. The nymph might get bigger at third day before molting to third instar.

Table 4.2 Morphological characteristics of second instar nymph of *Pentalonia nigronervosa* lived on rodent tuber

Plant sample…	Morphological characteristic			
	color	Body length (mm)	Body width (mm)	Instar period (days)
1	Pale brown	0.71	0.36	3
2	Pale brown	0.83	0.55	3
3	Pale brown	0.72	0.45	3
4	Pale brown	0.79	0.48	1
5	Pale brown	0.79	0.47	3
6	Pale brown	0.78	0.47	2
7	Pale brown	0.74	0.51	1
8	Pale brown	0.83	0.53	2
9	Pale brown	0.73	0.58	2
10	Pale brown	0.72	0.44	3
Total		7.64	4.84	25
Average		0.764	0.48	2.50
Standard deviation		0.01	0.02	0.26

4.1.1.3 Third Instar

Third instar of *P. nigronervosa* nymphs were reddish brown in colour, the size had getting bigger and the body shape little bit changed (slightly longer oval) and their legs became darker (Figure 4.3.)

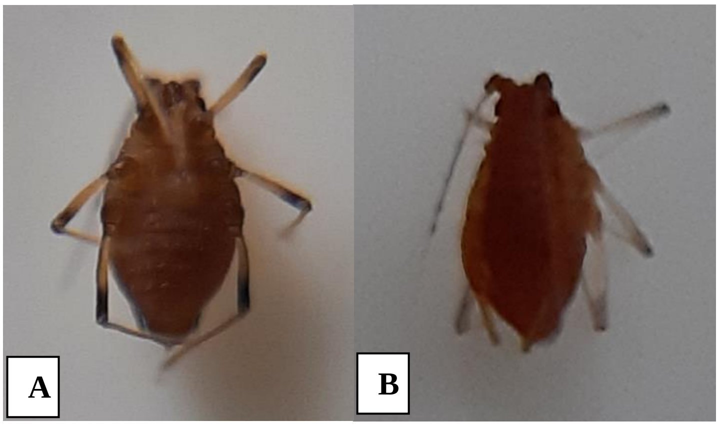


Figure 4.3. Banana aphid *Pentalonia nigronervosa* lived on rodent tuber (A) third instar, ventral, (B) third instar, dorsal

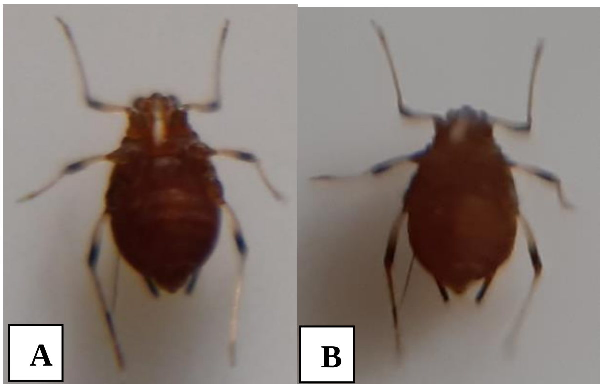
Body length of third instar of *P. nigronervosa* nymphs ranged from 0.83 to 0.97 mm and width ranged from 0.41 to 0.6 mm (Table 4.3). The measuring activity was conducted one day after molting from second to third instar nymph. The size of the nymphs might be different if the measurement was taken on the other days of third instar period which was 2.2 days on average.

Tabel 4.3 Morphological characteristics of third instar nymph of *Pentalonia nigronervosa* lived on rodent tuber

Plant sample…	Morphological characteristic			
	color	Body length (mm)	Body width (mm)	Instar period (day)
1	Reddish brown	0.87	0.41	3
2	Reddish brown	0.97	0.56	3
3	Reddish brown	0.85	0.5	1
4	Reddish brown	0.88	0.51	2
5	Reddish brown	0.87	0.52	1
6	Reddish brown	0.87	0.47	3
7	Reddish brown	0.83	0.55	2
8	Reddish brown	0.92	0.56	3
9	Reddish brown	0.86	0.6	2
10	Reddish brown	0.83	0.5	2
Total		8.75	4.64	22
Average		0.875	0.46	2.20
Standard deviation		0.01	0.02	0.25

4.1.1.4 Fourth Instar

Fourth instar of the nymph of *P. nigronervosa* lived on rodent tuber was dark brown in colour, the size has getting bigger than previous instar and the shape also slightly different more elongated. Legs was darker and the antennae were more obvious (Figure 4.4.)



Figurer 4.4. Banana aphid *Pentalonia nigronervosa* lived on rodent tuber; (A) fourth instar, ventral (B) fourth instar, dorsal

Body length of fourth instar of *P. nigronervosa* nymphs ranged from 1.21 to 1.37 mm and the width ranged from 0.53 to 7.7 mm (Table 4.4). The measuring activity was conducted one day after molting from third to fourth instar nymph. The size of the nymphs might be different if the measurement was taken on the other days of third instar period which was 1.6 days on average.

Tabel 4.4 Morphological characteristics of fourth instar nymph of *Pentalonia nigronervosa* lived on rodent tuber

Plant sample…	Morphological characteristic			
	color	Body length (mm)	Body width (mm)	Instar period (day)
1	Dark brown	1.3	0.53	2
2	Dark brown	1.21	0.64	1
3	Dark brown	1.33	0.69	2
4	Dark brown	1.31	0.67	1
5	Dark brown	1.35	0.73	2
6	Dark brown	1.37	0.77	1
7	Dark brown	1.31	0.65	2
8	Dark brown	1.22	0.6	2

9	Dark brown	1.34	0.7	2
10	Dark brown	1.4	0.75	1
Total		13.14	6.73	16
Average		1.314	0.67	1.60
Standard deviation		0.02	0.02	0.16

4.1.1.5 Imago

Imagoes of *P. nigronervosa* lived on rodent tuber was dark brown in colour and the size had reached their maximum. The body shape also slightly different from previous instars. All organs have been more obvious such as yes, antennae and legs (Figure 4.5.)

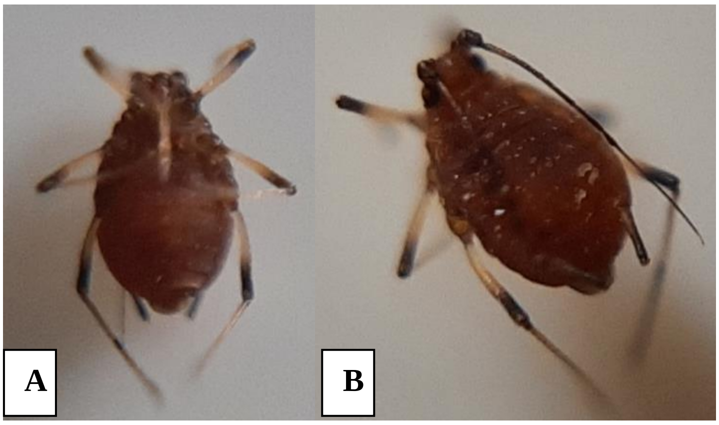


Figure 4.5. Banana aphid *Pentalonia nigronervosa* lived on rodent tuber; (A) imago, ventral, (B) Imago, dorsal

In this phase and in natural habitat, some of the imago of *P. nigronervosa* some would have wings (alate) and others remain wingless (apterous). However, we did not find any winged imago lived on rodent tuber. This could be caused by the condition in the experiment bioassay population of the aphid was kept small because for the accuracy of progeny calculation, newly born nymph was remove from the experimental plants and transfer to other plant outside the enclosed cup. Therefore, no need for imago to have wings because no urgency to leave the host. Body length of *P. nigronervosa ranged* from 1.4 to 1.7 mm and the width ranged from 0.93 to 0.99 mm. Average fecundity was 18.3 offspring per female with average reproductive period 8.2days and imago life span 12.5 days (Table 4.5.).

Tabel 4.5 Morphological and biological characteristics of imago of *Pentalonia nigronervosa* lived on rodent tuber

Plant sample	Morphological and biological characteristic							
	color	Body length (mm)	Bode width(mm)	Instar period (days)	Number of off-spring	Reproductive period	% alate	Imago lifespan (days)
1	Dark brown	1.42	0.97	13	15	6	0	13
2	Dark brown	1.4	0.94	10	13	6	0	10
3	Dark brown	1.44	0.97	15	17	10	0	15
4	Dark brown	1.41	0.95	13	25	10	0	13
5	Dark brown	1,42	0.99	16	15	12	0	16
6	Dark brown	1,45	0.99	10	19	7	0	10
7	Dark brown	1.42	0.93	11	17	7	0	11
8	Dark brown	1.44	0.94	13	22	8	0	13
9	Dark brown	1.47	0.99	9	19	6	0	9
10	Dark brown	1.46	0.97	15	21	10	0	15
Total		11.46	8.67	125	183	82	0	125
Average		1.4325	0.96	12.50	18.3	8.2	0	12.5
Standard deviation		0.01	0.01	0.76	1.16	0.68	0.00	0.76

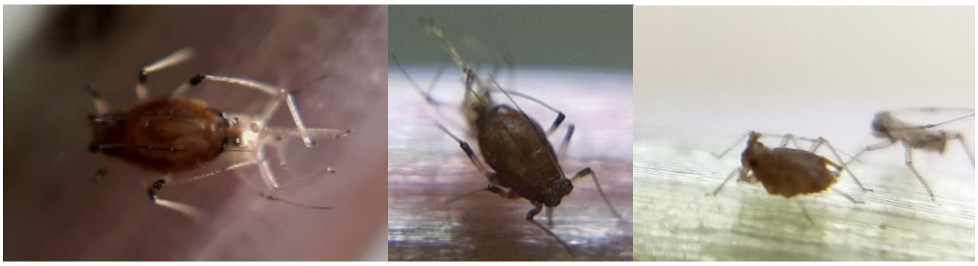


Figure 4.6. Banana aphid *Pentalonia nigronervosa* at molting process

Figure 4.7. Banana aphid *Pentalonia nigronervosa* gave birth to progeny

4.1.2. Environmental Data of Bioassay of *Pentalonia nigronervosa* Lived on Rodent Tuber

Bioassay of *P. nigronervosa* lived on rodent tuber was conducted in insectarium where temperature was maintained around 25^{o}C in order to match the need of *P. nigronervosa* to grow and breed. Variation of environmental data were as presented in Table 4.6.

Table 4.6. Environmental data in the room for bioassay of *Pentalonia nigronervosa* on Rodent Tuber

Date	Time					
	08.00		12.00		16.00	
	Tempera-ture (°C)	% RH	Tempera-ture (°C)	% RH	Tempera-ture(°C)	% RH
25 July 2021	21,9	57	23,8	60	25,7	64
26 July 2021	22	60	24	76	26	62
27 July 2021	25	76	27,5	87	26	86
28 July 2021	25,8	86	26,5	69	25,6	61
29 July 2021	26,2	87	30,1	59	26,5	60
29 July 2021	25,4	77	26,2	67	25,6	57
30 July 2021	25,1	83	26,4	66	25,5	59
31 July 2021	25,6	84	26	67	24,8	62
1 August 2021	25,2	79	25,6	69	26,4	65
2 August 2021	26,1	85	25,7	66	25,9	65
3 August 2021	26,3	87	26,7	72	25,1	57
4 August 2021	25,6	84	26	77	24,8	64
5 August 2021	25,1	58	25,5	75	25,4	64
6 August 2021	24,3	65	26	75	25,4	66
7 August 2021	24,5	65	25,3	53	24,2	63
8 August 2021	25,2	73	26,2	64	25,1	68
9 August 2021	25,6	62	26,3	73	26,1	54
10 August 2021	24,9	58	26,2	59	26	54
11 August 2021	26,2	82	26,9	65	26,4	56
12 August 2021	24,1	58	26,4	73	25,1	72
13 August 2021	22,8	50	25,2	87	24,3	64
14 August 2021	24,9	84	25,5	63	24,2	65
15 August 2021	23,4	59	26,2	57	24,5	64
16 August 2021	25,8	84	26,1	50	25,7	47
17 August 2021	26,4	89	28	59	26,1	76
18 August 2021	25,2	66	26,4	65	25,8	65
19 August 2021	25,3	64	27,3	61	27,8	59
20 August 2021	25,9	62	26,1	72	26,5	70
21 August 2021	23,4	49	26,8	58	24,3	57
22 August 2021	23,2	51	25	60	24,2	54
23 August 2021	23,8	56	24,3	55	23	57
24 August 2021	25,4	92	24	87	23	59
25 August 2021	24,2	58	28	88	28,2	88
26 August 2021	24,5	55	24	63	23,7	51

4.1.3. Field Experiment Data

Symptom of BBTV infection on banana inoculated with BBTV using *P. nigronervosa* appeared for the first time at 14 days after inoculation. The first symptoms appeared on young leaves showing slight yellowing and vein banding (Figure 4.8.)

Figure 4.8. Banana leaf inoculated with BBTV using vector *P.* nigronervosa; (A) before being infected by BBTV, (B), first symptom of BBTV infection on inoculated banana

Results of observation showed that rodent tuber could not affect the efficiency of BBTV transmission by *P.* nigronervosa shown by the fact that banana plant inoculated with BBTV by viruliferous aphid stayed for up to 72 hours in rodent tuber still produced BBTV infection symptom. Data recorded and analyses are presented in appendices, and the average are presented in Table 4.7.

Tabel 4.7. Incubation of BBTV on banana inoculated by *P. nigronervosa* passed on rodent tuber before virus transmission

Treatment	Average incubation period Ke (Hari)
P1 (0 hour passing period)	10.33±0.62
P2 (24 hours passing period)	19.46±0.49
P3 (48 hours passing period)	10.13±0.38
P4 (72 hours passing period)	11.73±0.48
F count	0.55
F table	3.49
LSD 5%	nsd

*nsd = not significantly different

4.1.4. Disease Intensity

BBTV infection was begun with the appearance of wrinkling on infected leave and the leave turn into pale yellow and the vein getting more obvious (vein banding) (Figure 4.9).

Figure 4.9. Banana plant showing advance phase symptom of BBTV infection

Rerata serangan penyakit BBTV pada ke empat perlakuan dapat dilihat pada tabel 4.8 yang mana jumlah F hitung sebesar 0.54 dan F tabel sebesar 3.49. yang berarti hasilnya adalah tidak berbeda nyata (tn) hal ini bisa di pengaruhi oleh beberapa faktor lingkungan yakni suhu, kelembaban, cuaca dan keberadaan musuh alami.

Table 4.8. Average disease intensity of BBTV on banana inoculated by *P. nigronervosa* passed on rodent tuber before virus transmission

Treatment level	Disease incidence
P1 (0 hour passing period)	66±10.54
P2 (24 hours passing period)	59±6.66
P3 (48 hours passing period)	59±6.66
P4 (72 hours passing period)	59±6.66
F count	0.54
F table	3.49
LSD 5%	nsd

*nsd = not significantly different

4.2. Discussion

At the time of transferring banana aphid to rodent tuber for reproduction on the plant, we used forth instar nymphs. The aphid could adapt themselves to rodent tuber and produced progenies which were then used for bioassay experiment. They started to reproduced 24 hours after being transferred to rodent tuber. This could be promoted by the environment conditions arranged to be suitable for the aphid.

Every instar had its own characteristics such as size and color. First instar was whitish brown in color with body length ranged from 0.61 to 0.69 mm Second instar was pale brown in color with body length ranged from 0.71 to 0.83 mm. Third instar was reddish brown in color with average body length 0.87 mm, and fourth instar was dark brown in color with average body length 1.31 mm. At the phase of imago, the color was the same as the fourth instar, dark brown, with average body length 1.43 mm. In its main host, some imagoes were winged but in this research, we did not find any winged imago. Based on the result of research conducted by Muthia (2017) banana aphid *P. nigronervosa* could develop very well on Araceous crops, one of them was rodent tuber. She reported that *P. nigronervosa* could reproduce 12 hours after being transfer to host for virus transmission.

As other aphids, banana aphid *P. nigronervosa* do not lay eggs and reproduce fully parthenogenetically. All of the colony members are female (Rahmah, 2018). However, the number of progenies produced by *P. nigronervosa* female lived on rodent tuber was not as much as those laid by the aphid lived on banana and other Araceous plants. This could be caused by something related to the morphology of rodent tuber. Rodent tuber is smaller compare to other plant species in the family Araceae so that the plant become senescence more quickly and the aphid lived on the plant faced the situation of lacking water and nutrients (Sudiono and Yasin, 2006).

Population of banana aphid could also be affected by two factors i.e. Biotic and abiotic factors. The main abiotic factors were temperature and humidity. Optimum temperature for *P. nigronervosa* to grow and develop ranges from 15 to 25ºC with humidity 80% (Robson and Wright, 2007). In this research, temperature and humidity in insectarium ranged from 23 to 20°C with RH ranged from 50 to 88%. Biotic factor influencing the aphid population included nutrition source and natural enemies. One of nutritional sources which was not suitable to the aphid was the low contain of water in the host and the hardness of host surface (Suparman, Nurhayati and Setyawaty, 2011). In the case of rodent tuber, the plant contained enough water and nutrient and soft surface which was preferred by aphids.

Transmitting BBTV using *P. nigronervosa* was conducted in the evening so the environment temperature was not too high. The result show3d that the fastest incubation period was 14 days for which, treatment P1 (no passing period on rodent tuber) showed the shortest incubation period. The symptom appeared started by the formation of wrinkle leaf dan changed color to pale yellow. Advanced disease development was indicated by stunting symptom of the infected plant. Most infected plants failed to produce fruit. In all treatment levels, there were 23 plants did not show any symptom and 37 plants showed virus infection. Among 23 plants without symptom, 5 plant were from treatment P1, 6 from P2, 6 plants from P3 and the other 6 plant were from P4. There was no significant different among treatment levels, but the average proportion of symptomless plant was relative high indicating that average transmission efficiency was 61.67 %, meant that even though the passing viruliferous *P. nigronervosa* on rodent tuber could reduce the efficiency of BBTV transmission. However, rodent tuber was proved to be one of alternative host of *P. nigronervosa*.

At the time of transmitting *P. nigronervosa to healthy plant in the field*, it was rainy season so we had to enclose the plant using transparent plastic bag to [protect aphid from being washed by rain. The plastic cover was removed during the day only if there was no rain. After 6bdays, the plastic covers were totally removed. Symptom on P1 appeared at 14 days after transmission and the longest was 17 days after transmission. Symptom on P2 treatment appear at 14 to18 days after transmission. Symptom on P3 treatment appeared at 24 to 19 days after transmission and at P4 treatment, the first symptom appeared at 14 to 21 days after transmission. P1 showed the fastest incubation period might be because the viruliferous aphid did no pass any period on rodent tuber compared to other treatment levels.

CHAPTER 5
CONCLUSION AND SUGGESTION

5.1. Conclusion

Based on the bioassay experiment results, *P. nigronervosa* could live and breed normally on rodent tuber with morphological and biological characteristics not too much different from those lived on banana as its main host.

No alate imago was found on rodent tuber, but it did not mean that the aphid could not form wings on rodent tuber. The aphid did not form alate imago because the population was kept low by transferring newly born nymph to easy the progeny counting.

Based on field experiment it was found that rodent tuber did not significantly affect the efficiency of BBTV transmission by *P. nigronervosa.*

5.2. Suggestion

Rodent tuber cam be the alternative host for *P. nigronervosa and* avoid planting the plant surrounding banana cultivation is recommended.

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APPENDICES

Appendix 1. Table of incubation period and disease incidence resulted by four treatment levels.

Treatment level	Repli-cation	Plant number	Incubation period	Disease incidence	Average incubation period	Average disease incidence
P1		1	15	100		
	1	2	16	100		
		3	0	0	10.33	66.66
		1	14	100		
	2	2	15	100		
		3	0	0	9.66	66.66
		1	16	100		
	3	2	17	100		
		3	16	100	16.33	100
		1	0	0		
	4	2	17	100		
		3	14	100	10.33	66.66
		1	0	0		
	5	2	15	100		
		3	0	0	5	33.33
P2		1	15	100		
	1	2	14	100		
		3	0	0	9.66	66.66
		1	0	0		
	2	2	16	100		
		3	18	100	11.33	66.66
		1	0	0		
	3	2	18	100		
		3	15	100	11	66.66
		1	0	0		
	4	2	17	100		
		3	15	100	10.66	66.66
		1	0	0		
	5	2	14	100		
		3	0	0	4.66	33.33
P3		1	15	100		
	1	2	14	100		
		3	0	0	9.66	66.66
	2	1	16	100		
		2	18	100	11.33	66.66

		3	0	0		
		1	17	100		
	3	2	16	100		
		3	0	0	11	66.66
		1	19	100		
	4	2	18	100		
		3	0	0	12.33	66.66
		1	19	100		
	5	2	0	0		
		3	0	0	6.33	33.33
P4		1	20	100		
	1	2	20	100		
		3	0	0	13.33	66.66
		1	0	0		
	2	2	19	100		
		3	0	0	6.33	33.33
		1	18	100		
	3	2	0	0		
		3	21	100	13	66.66
		1	20	100		
	4	2	0	0		
		3	18	100	12.66	66.66
		1	21	100		
	5	2	0	0		
		3	19	100	13.33	66.66

Appendix 2. Table of Incubation period data

Treatment level	Incubation period					Total	Average
	1	2	3	4	5		
P1	10.33	9.66	16.33	10.33	5	51.65	10.33
P2	9.66	11.33	11	10.66	4.66	47.31	9.462
P3	9.66	11.33	11	12.33	6.33	50.65	10.13
P4	13.33	12.66	13	6.33	13.33	58.65	11.73
Total	42.98	44.98	51.33	39.65	29.32	208.26	41.652

Appendix 33. Table of incubation period data according to block

Treatment level	Incubation period					total	average
	1	2	3	4	5		
P1	10.45	10.28	11.69	10.45	8.81	51.68	10.34
P2	10.28	10.69	10.61	10.53	8.67	50.78	10.16
P3	10.28	10.69	10.61	10.91	9.30	51.80	10.36

P4	11.12	10.98	11.05	9.30	11.12	53.57	10.71
Total	42.14	42.64	43.96	41.20	37.90	207.84	

Appendix 4 4. Table of analysis of variance

Variance	Degree of freedom	Sum of square	Mean square	F Count	F Table
Treatment	3	0.82	0.27	0.55	3.49
Block	4	5.20	1.30	2.62	3.26
Error	12	5.94	0.50		
Total	19	11.96			

Appendix 5. Table of disease incidence of BBTD

Treatment level	Disease incidence (%)					Total	Average
	1	2	3	4	5		
P1	66.66	66.66	99.91667	66.66	33.33	333.23	66.65
P2	66.66	66.66	66.66	66.66	33.33	299.97	59.99
P3	66.66	66.66	66.66	66.66	33.33	299.97	59.99
P4	66.66	33.33	66.66	66.66	66.66	299.97	59.99

Appendix 6. Table of Arcsin transformation of disease incidence

Treatment level	Disease Incidence (%) for block					Total	Average
	1	2	3	4	5		
P1	54.731	54.731	88.345	54.731	35.262	287.80	95.934
P2	54.731	54.731	54.731	54.731	35.262	254.18	84.729
P3	54.731	54.731	54.731	54.731	35.262	254.18	84.729
P4	54.731	35.262	54.731	54.731	54.731	254.18	84.729
Total	218.92	199.45	252.540	218.926	160.518	1050.36	

Lampiran 7. Table ANOVA of BBTV disease incidence

Variance	Degree of freedom	Sum of square	Mean square	F count	F Table
Treatment	3	169.4874	56.4958	0.543872	3.490295
Block	4	1132.144	283.0361	2.724723	3.259167
Error	12	1246.524	103.877		
Total	19	2548.156			

Appendix 7. Field experiment

Appendix 8. transferring *P. nigronervosa* to healthy banana

Appendix 9. Cutting banana sucker to produce uniform young shoot

Appendix 10. Rodent tuber

Appendix 11. Observation to determine incubation period

Appendix 12. Bioassay *P. nigronervosa* on rodent tuber

Appendix 13. Mild symptom at the beginning of BBTV infection

Appendix 13. Symptom of advance infection of BBTV

Appendix 14. Enclosing banana after BBTV transmission by *P. nigronervosa*