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Natural enemies of *Pentalonia nigronervosa*, vector of Banana Bunchy Top Virus

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Abstract. *Tricahyati T, Suparman, Irsan C. 2022. Natural enemies of* Pentalonia nigronervosa*, vector of Banana Bunchy Top Virus. Biodiversitas 23: 3675-3684. Pentalonia nigronervosa* (Coquerel) is an important pest of banana. Instead of sucking liquid from banana phloem, *P. nigronervosa* also play an important role in transmitting Banana Bunchy Top Virus (BBTV) from infected banana to healthy ones. If efforts are not made to control the aphid, existence of *P. nigronervosa* can increase in the banana field. The objective of this research was to find out the natural enemies of *P. nigronervosa* which may play significant role in controlling the aphid naturally. The results showed that natural enemies were in the forms of predator, parasitoid and pathogenic fungi. A total of 22 species of predator belonged to 5 families of insect, namely Coccinellidae, Forficulidae, Chelisochidae, Reduviidae and Syrphidae, and 4 families of Arachnida i.e. Oxyopidae, Araneidae, Salticidae, and Philodromidae. The result exhibited that one parasitoid belonged to family Brachonidae, and one hyper-parasitoid belonged to family Encrytidae. Furthermore, it was also observed that 3 species of entomopathogenic fungi infected aphids. The fungi belonged to genera *Aspergillus* and *Beauveria*. Each predator had different predation capacity, the highest was that of Forficula auricularia (23.67 ± 3.05) and Scymnus sp. (23.67 ± 1.52). Parasitoid Lipolexis bengalensis could parasitize *P. nigronervosa* at relatively high parasitic ability of 4.67 ± 23.65. The finding of various natural enemies of *P. nigronervosa* in South Sumatra could be considered as an alternative way to control the aphid and reduce the transmission rate of BBTV in the province. The use of natural enemies has no residual effects on the environment and is relatively cheaper compared to other control measurements.

Keywords: Entomopathogenic fungi, hyper-parasitoid, natural enemy, parasitoid, *Pentalonia nigronervosa*, predator

INTRODUCTION

Banana (Musa spp.) is cultivated everywhere in the world, especially in tropical countries such as Indonesia (Lestari and Hidayat 2020). In the cultivation of banana crops, farmers frequently find various diseases which cause severe damage to the crop. The diseases can be caused by fungi, bacteria, nematodes, or viruses (Molina et al. 2019). Some viruses can infect banana, including Banana Bunchy Top Virus (BBTV), Banana Streak Virus (BSV), Banana Bract Mosaic Virus (BBrMV) (Som et al. 2018) and Banana Xanthomonas Wilt (BXW) (Wanjiku et al. 2021). BBTV causes very important disease on banana called Banana bunchy top disease (Halbert and Baker 2015). BBTV infects various banana cultivars such as Lady Finger, Cardaba, Cavendish and others (Qazi 2016; Latifah et al. 2021). Banana plants infected with BBTV produce either no fruit or very poor quality fruit so that the disease has significant economic effect on the banana industry. Infected banana leaves are narrow, fragile, and close to each other to make a bunchy appearance (Sairam et al. 2020). BBTV is found almost everywhere in Indonesia especially in Sumatera Island (Chiaki et al. 2015). It has been reported that West Sumatra, Bengkulu, and South Sumatra are the major provinces of BBTV, which can infect various species of banana, such as *Musa acuminata,* i.e. malacensis, longipetiolata, halabanensis, sumatrana, and *Musa* spp. (Rahayuniati et al. 2021). In these areas, *Pentalonia nigronervosa* has been found associated with several banana cultivars such as cv. Kepok, cv. Raja, cv. Mas, and cv. Cavendish (Rahmah et al. 2021). BBTV has a greater impact on commercial banana cultivars then on wild cultivars (Arubi et al. 2021). The most serious problem of BBTV is the transmission of the virus by its main vector, *P. nigronervosa* (Maharani and Hidayat 2019). Another vector of BBTV is *P. caladii*. However, *P. nigronervosa* is more efficient in transmitting the virus and distributed more widely than *P. caladii* (Watanabe et al. 2013). Banana plants are the best host for the growth of *P. nigronervosa* (Robson and Wright 2007a). However, there are other plant species in which *P. nigronervosa* can live and develop, especially those belonging to Musaceae, Zingiberaceae, and Araceae (Bhadra and Agarwala 2010). Plant species identified as alternative host for *P. nigronervosa* include pink ginger, elephant ear, cardamom, tomato, and taro (Capinera 2008). In Indonesia, there are 112 aphid species infesting agriculture, 23 species of them are important pests, of which 21 species are plant virus vectors (Maharani and Hidayat 2019), and *P. nigronervosa* is one of the most important virus vectors (Yele and Poddar 2019). *Pentalonia nigronervosa* is widespread in tropical and subtropical areas (Mille et al. 2020) and its population increases significantly every summer (Niyongere et al. 2012). Integrated pest management is a key to suppressing pests and diseases in the field (Sarwar 2011) including control of BBTV. The control of BBTV is quite difficult if organized on a small agricultural scale (Ocimati et al. 2021). Several methods have been implemented to control BBTV, including the use of resistance cultivar, cultural technique, biological control, and chemical control (Sandhi and Reddy 2021). The use of virus free suckers, entomopathogen, predator and parasitoid have also been applied to control the virus (Kakati and Nath 2019). In addition, predators, parasitoids, entomopathogenic fungi and hyper-parasitoid are also used as good natural enemies to control aphids (Rafi et al. 2010; Boivin et al. 2012). Scymnus sp. is an aphid predator originally from Thailand and was introduced in December 2000. Currently, this predator has been massively reared and used experimentally to control aphid. It was reported that *Scymnus* sp. could effectively control aphid species (Culliney et al. 2003). Besides *Scymnus* sp., other predators such as *Adalia bipunctata* have also been reported as a good predators of *P. nigronervosa* (Chaudhary and Singh 2012). Other parasitoids that have been reported to be natural enemies of *P. nigronervosa* are *Ephedrus plagiator, Lysiphlebus fabarum*, and *Aphidius transcaspicus* (Wang and Messing 2006; Völkl et al. 2015). Female parasitoids parasitize aphid without individual choice and have high parasitic ability (Völkl and Stadler 1991). Parasitoid belongs to genus Lipolexis and has also been reported parasitizing aphids belonging to genus Aphis (Kocić et al. 2020). Persad et al. (2004) conducted a field study and found many aphids parasitized by *Lipolexis oregmae*. In the field, parasitized aphids on citrus leaves turn into mummy in 8 to 10 days (Persad et al. 2007). Aphid mummies have various colors depending on the parasitized aphids' host (Singh et al. 2007). A number of entomopathogenic fungi have also been identified as pathogen of aphid, namely *Beauveria bassiana, Metarhizium anisopliae,* and *Verticillium lecanii* (Vu et al. 2007). According to González-Mas et al. (2021), entomopathogenic fungi could effectively control various insect in the field and the environment. The pathogen could infect larvae and pupae of the targeted host insect (Trizelia et al. 2017).

The natural enemies of *P. nigronervosa* in Indonesia have not been thoroughly identified. The objective of this research was to identify predator, parasitoid, and fungal pathogen of *P. nigronervosa* with the hope that all of the identified natural enemies can be incorporated in the control of banana aphid as the main vector of banana bunchy top virus, causal agent of banana bunchy top disease.

MATERIALS AND METHODS

Study area

The research was conducted in the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indonesia, from October to December 2021. The collection was carried out in the period of October-December because an increase in the number of *P. nigronervosa* colonies was found on banana plants around the observation area, which was thought to be due to the arrival of summer. All natural enemies identified were collected from banana cultivation in the Regencies of Ogan Ilir and Muara Enim, South Sumatra, Indonesia. The natural enemies collected from the areas were predator, parasitoid, hyper-parasitoid, and entomopathogenic fungi as specified in Table 1.

Procedures

Collection of aphid mummies and predator arthropods

The collection of aphid predators and aphid mummies from the field was conducted according to the procedure of Biale et al. (2017). The larva or imago of each found predator was taken using paint brush and put into a vial, while aphid mummies were collected by cutting parts of plant containing aphid mummies. Samples of predators and aphid mummies were brought to laboratory for further treatments. Mummies were kept in certain containers until their imagoes emerged for identification. All predators and aphid mummies were collected from banana plants.

Parasitoid rearing

Aphid mummies collected from the field were placed in topless. After the parasitoids emerged from the mummies, they were placed in a plastic cylinder and its top was covered with a cheese cloth to facilitate air movement (Utami et al. 2014).

Preparation of Pentalonia nigronervosa

Pentalonia nigronervosa collected from banana cultivation was reared in the laboratory using young banana suckers placed in a plastic cylinder with a cheese cloth at the top surface.

Table 1. The characteristics of natural enemies of *Pentalonia nigronervosa*

Characteristic	Predator	Parasitoid	Hyper-parasitoid	Entomopathogenic Fungi
Taxon	Invertebrate	Insect	Insect	Fungi
Host	Arthropod	Insect	Insect	Insect
Host specificity	Less specific	Specific	Specific	Specific
Host size	Same or smaller	Similar	Bigger	Bigger
Attacking phase	Pre imago and imago	Pre imago	Pre-imago	Pre-imago
Host number	A lot	One	One	A lot
Killing potential	Kill immediately	Kill after few days	Kill after few days	Kill after few days

Predation capacity assessment

The predation ability of each identified predator against *P. nigronervosa* was assessed by placing the predator in a plastic container containing 30 nymphs of 2nd instar of *P. nigronervosa* for 2 hours and were used in three replicates (Jaworski et al. 2013), and then counted the number of the aphid nymphs preyed by each predator who had been fasted for 24 hours (Nelly 2012).

Parasitic Ability assessment

Parasitoids emerged from collected mummies were placed in a plastic container containing 2-4 instar of *P. nigronervosa* and were used in six replicates with 90 health *P. nigronervosa* per replication. 14 days later, observations were made to determine whether the parasitoid could parasitize *P. nigronervosa* nymphs or not, shown by the formation of mummies (Völkl et al. 2015).

Identification of predator and parasitoid

All predators and parasitoids required for identification were placed in a 70% alcohol. Predators and parasitoids were identified by observing the morphological characteristic, such as form, size and color of body and wings (Zu et al. 2018; Kocić et al. 2020). All specimens were photographed for documentation. The identification process was supervised by Dr. Chandra Irsan, an insect taxonomist at Sriwijaya University.

Identification of entomopathogenic fungi

The fungi infecting *P. nigronervosa* in the field were isolated and inoculated to healthy *P. nigronervosa* to observe the development of infection on the aphid. The fungal pathogen was identified in the laboratory by examining the fungal characteristics under a microscope. Observation under microscope was made specially to characterize the mycelium and spores of fungi. The identification process was conducted under supervision by Dr. Suparman SHK, a mycologist and plant pathologist at Sriwijaya University.

Data analysis

Data analysis was conducted by using a statistic application named RStudio, data was also presented in the form of a table, graphic and photograph.

RESULTS AND DISCUSSION

Predator of *Pentalonia nigronervosa*

The result showed that a total of 22 species of arthropod were identified that played a role as predator of *P. nigronervosa* (Table 2). Identified predators belonged to classes Insecta and Arachnida. There were 4 orders of Insect consisting of 5 families and 11 species and class Arachnida contained 1 order and 4 families comprised of 11 species (Figure 1). The predatory ability of all the identified predators was different and the highest was recorded by *Forficula auricularia* (23.67 ± 3.05) and *Scymnus* sp. (23.67 ± 1.52), while the lowest was that of *Plexippus paykulli* (3.66 ± 1.52). Predator belonged to family Coccinellidae generally had relatively high predation capacity against *P. nigronervosa.* Besides *Scymnus* sp., other predators also had significantly high predation capacity, such as *Menochilus sexmaculatus* (20.67 ± 2.08) and *Coelophora* sp. (20.67±2.51), *Coccinella transversalis* (20.33±1.52), *Verania lineata* (18.67 ± 1.52), *Micraspis discolor* (16.33 ±1.52) and *Micraspis hirashimai* (13.00 ± 2.00) (Table 2). Two predator species were identified as the members of order Dermaptera. One species belonged to the family Forficulidae named *Forficula Auricularia*, which had a prediction ability of (23.67±3.05), and another belonged to the family Chelisochidae with species name *Chelisoches* sp. with a predation capacity of 14.33 ± 1.52. Another predator was identified as a member of order Diptera, family Syrphidae, namely *Dioprosopa* sp. which had a predation capacity of 15.00 ± 2.00. The predator was also identified as a member of order Hemiptera, family Reduviidae, namely *Harpactorinae* sp. which had a predation capacity of 8.00 ± 1.00. It was also observed that predators of *P. nigronervosa* were identified as the members of Arachnida or spider, order Araneae. Eleven families of this order were identified as the predator of banana aphid. The highest (8.67 ± 2.51) predation capacity of spider was recorded in *Oxyopes salticus* Hentz, followed by *Neoscona* sp. (8.00 ± 1.00), *Naphrys* sp. (7.66 ± 1.52), *Philodromus* sp., (B) (7.33 ± 1.52), *Thiania* sp., (7.33 ± 0.57), *Philodromus* sp., (A) (7.00 ± 1.00 g), *Habronattus* sp., (6.33 ± 2.08), *Menemerus bivittatus* (5.67 ± 0.57), *Hasarius adansoni* (5.33 ± 2.08), and *Trite* sp., (5.33 ± 1.52) (Tabel 2).

Parasitoid of *Pentalonia nigronervosa*

A species of parasitoid was identified to parasitize *P. nigronervosa*. The identified parasitoid was *Lipolexis bengalensis* Tomanovi'c and Koci'c (Hymenoptera: Braconidae) (Table 3, Figures 2 and 3). In parasitic ability assessment, it was found that the parasitoid could parasitize up to 98% of given banana aphids. The color of parasitized *P. nigronervosa* turned into golden brown and remained in a group at the lower part of the host plant (Figure 4).

Entomopathogenic fungi infecting *Pentalonia nigronervosa*

The results revealed that there were three different entomopathogenic fungi infecting banana aphid *P. nigronervosa*. These fungi were *Aspergillus* sp. (a), *Aspergillus* sp. (b), and *Beauveria* sp. Fungal colonies developed in the body of *P. nigronervosa* with different colors and sporulation. Aphids infected with *Aspergillus* sp. (a) developed yellow fungal colony, while those infected with *Aspergillus* sp. (b) developed white fungal colony, and on banana aphids infected with *Beauveria* sp., also developed white colony (Figure 5).

Table 2. The average predatory capacity (predation rate) of the predator of *Pentalonia nigronervosa* given 30 aphids for 2 hours

Classs/ Order/ Family	Species	Mean ± SE
Insecta/Coleoptera/Coccinellidae	*Verania lineata*	18.67 ± 1.52 abcd
Insecta/Coleoptera/Coccinellidae	*Micraspis discolor*	16.33 ± 1.52 bcde
Insecta/Coleoptera/Coccinellidae	*Menochilus sexmaculatus*	20.67 ± 2.08 ab
Insecta/Coleoptera/Coccinellidae	*Coccinella transversalis*	20.33 ± 1.52 abc
Insecta/Coleoptera/Coccinellidae	*Scymnus* sp.	23.67 ± 1.52 a
Insecta/Coleoptera/Coccinellidae	*Coelophora* sp.	20.67 ± 2.51 ab
Insecta/Coleoptera/Coccinellidae	*Micraspis hirashimai*	13.00 ± 2.00 ef
Insecta/Dermaptera/Forficulidae	*Forficula auricularia*	23.67 ± 3.05 a
Insecta/Dermaptera/Chelisochidae	*Chelisoches* sp.	14.33 ± 1.52 de
Insecta/Hemiptera/Reduviidae	*Harpactorinae* sp.	8.00 ± 1.00 fg
Insecta/Diptera/Syrphidae	*Dioprosopa* sp.	15.00 ± 2.00 cde
Arachnida/Araneae/Oxyopidae	*Oxyopes salticus*	8.67 ± 2.51 fg
Arachnida/Araneae/Araneidae	Neoscona sp.	8.00 ± 1.00 fg
Arachnida/ Araneae/Salticidae	*Naphrys* sp.	7.66 ± 1.52 fg
Arachnida/ Araneae/Salticidae	*Thiania* sp.	7.33 ± 0.57 g
Arachnida/ Araneae/ Salticidae	*Menemerus bivittatus*	5.67 ± 0.57 g
Arachnida/ Araneae/ Salticidae	*Hasarius adansoni*	5.33 ± 2.08 g
Arachnida/ Araneae/ Salticidae	*Plexippus paykulli*	3.66 ± 1.52 g
Arachnida/ Araneae/ Salticidae	*Habronattus* sp.	6.33 ± 2.08 g
Arachnida/ Araneae/ Salticidae	*Trite* sp.	5.33 ± 1.52 g
Arachnida/ Araneae/ Philodromidae	*Philodromus* sp. (A)	7.00 ± 1.00 g
Arachnida/ Araneae/ Philodromidae	*Philodromus* sp. (B)	7.33 ± 1.52 g
F Hitung	43.25	
BNJ 5%	5.474	

Note: Figures followed by the same letter are not significantly different according to LSD 0.05

Table 3. Parasitic ability of *Pentalonia nigronervosa* parasitoid and its hyper-parasitoid

Order/ Family	Species	Role	Hosts	Average		
				No. of given *Pentalonia nigronervosa*	No. of mummies	No. of adult emergence
Hymenoptera/ Brachonidae	*Lipolexis bengalensis*	Parasitoid	*Pentalonia nigronervosa*	90	45	33
Hymenoptera/ Encrytidae	*Microterys angustus*	Hiperparasitoid	*L. bengalensis*	45	12	2

Discussion

A total of 22 species of *P. nigronervosa* predator were identified, including 1 species of parasitoid, 1 species of hyper-parasitoid, and 3 species of entomopathogenic fungi. Most identified predators were spiders and the rest were insects. The identified parasitoid and hyper-parasitoid belonged to the order Hymenoptera, and of the 2 identified entomopathogenic fungi one belonged to the genus *Aspergillus* and belonged to genus *Beauveria*. Seven of 22 identified predators were the members of family Coccinellidae. According to Chaudhary and Singh (2012), aphid predators are dominated by insects belonging to the family Coccinellidae, Syrphidae, Chrysopidae, and Hemerobiidae. Predators of *P. nigronervosa* generally showed the same predation capacity, but larval phase of predators such as *Scymnus* sp. tent to prey more than their adults. Predator larvae have higher predation capacity. Scymnus larvae tent to prey more, which means they needed more nutritious food for their larval development. Therefore, the use of *Scymnus* larvae has been reported to be effective in controlling *Aphis gossypii* (Bouvet et al. 2019). Larvae of *Scymnus syriacus* performed higher predation capacity than their male and female imagoes (Moradi et al. 2020). According to Hamback et al. (2021), third instar larvae of *Scymnus* had highest predation capacity compared to the other stadia of predator. *Scymnus flavicollis* was also reported as the banana aphid's main predator (Biale et al. 2017). *Sycmnus* has also been found as predator of insects living on other plants species such as citrus and *Ziziphus* (Najajrah et al. 2019). Generally, predators feed on various arthropods, as far as the prey arthropods have smaller body size than the predators themselves. Based on their predation capacity, the use of Coccinellidae predators has been promoted to preventively control population development of various aphids (Belyakova and Polikarpova 2020). Instead of feeding on aphis, Coccinellidae predators have also been reported to effectively reduce the population of *Dactylopius opuntiae* by preying on their eggs (Aalaoui et al. 2020). In the present investigation, several identified spiders preyed on species *P. nigronervosa* even though their predation capacity was lower than that of predacious insects. However, *Philodromus* sp. Has a high predation capacity against *Cacopsylla pyri* and *C. pyricola* (Gajski and Pekár 2021).

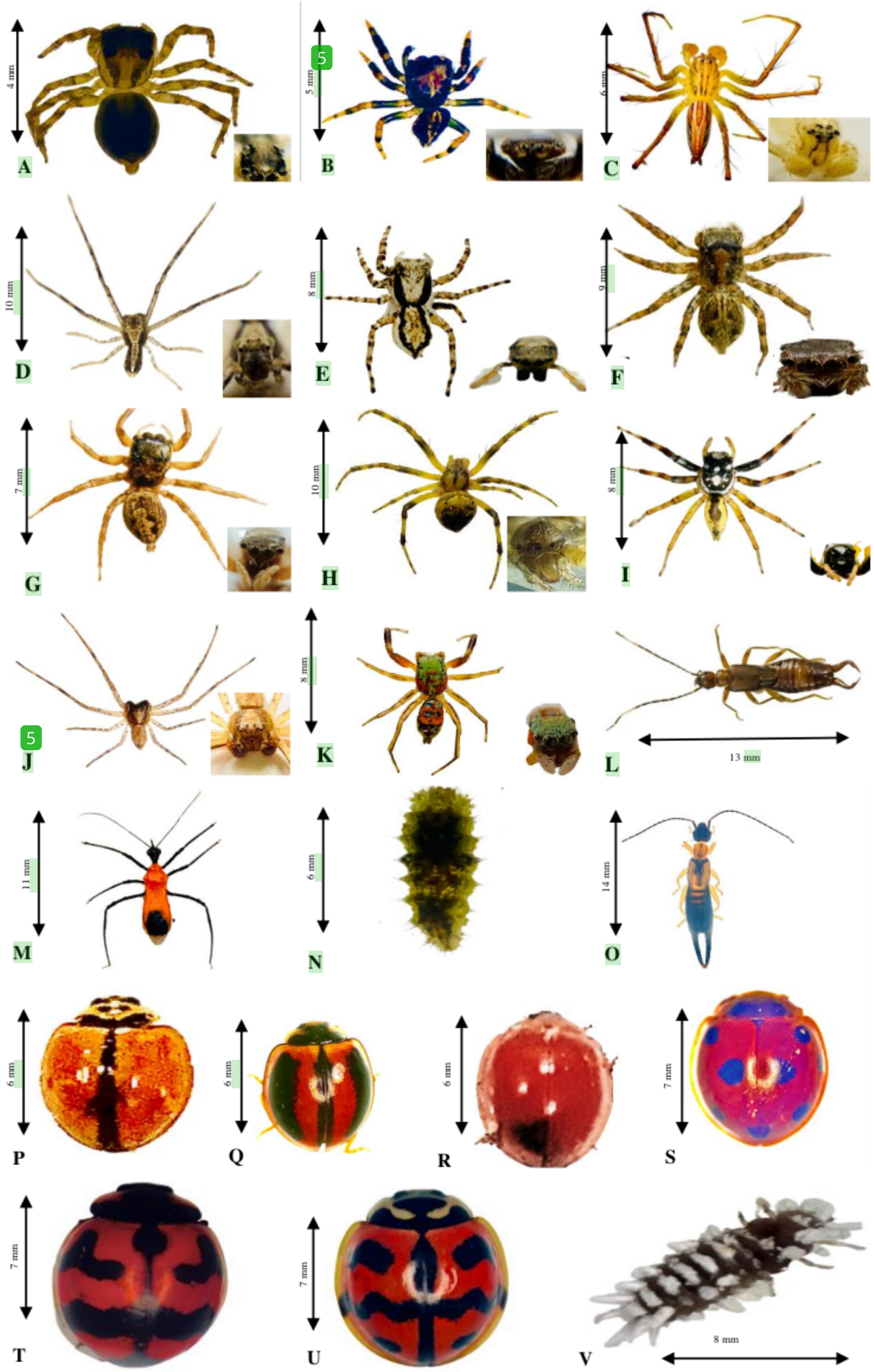

Figure 1. Predators of *Pentalonia nigronervosa*: A. *Naphrys* sp., B. *Thiania* sp., C. *Oxyopes salticus*, D. *Philodromus* sp., E. *Menemerus bivittatus*, F. *Plexippus paykulli*, G. *Hasarius adansoni*, H. *Neoscona* sp., I. *Habronattus* sp., J. *Trite* sp., K. *Philodromus* sp., L. *Chelisoches* sp., M. *Harpactorinae* sp., N. *Dioprosopa* sp., O. *Forficula auricularia*, P. *Micraspis discolor*, Q. *Verania lineata*, R. *Micraspis hirashimai*, S. *Coelophora* sp., T. *Coccinella transversalis*, U. *Menocchilus sexmaculatus*, V. *Scymnus* sp.

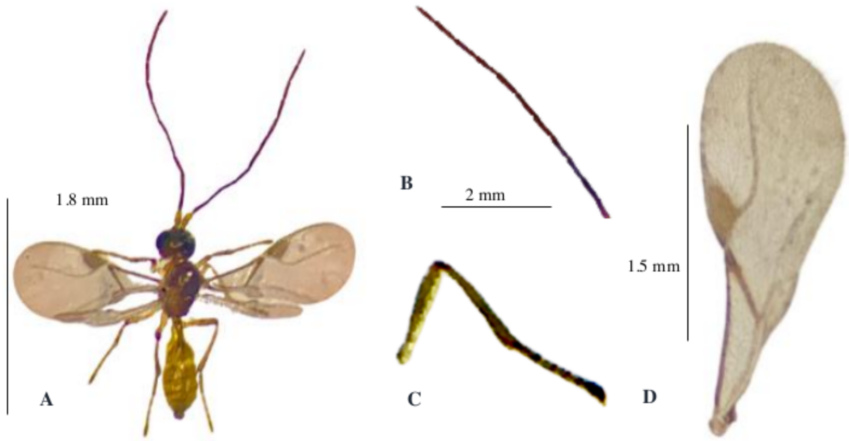

Figure 2. Morphology of *Lipolexis bengalensis*: A. full body, B. antena, C. Leg, D. wing

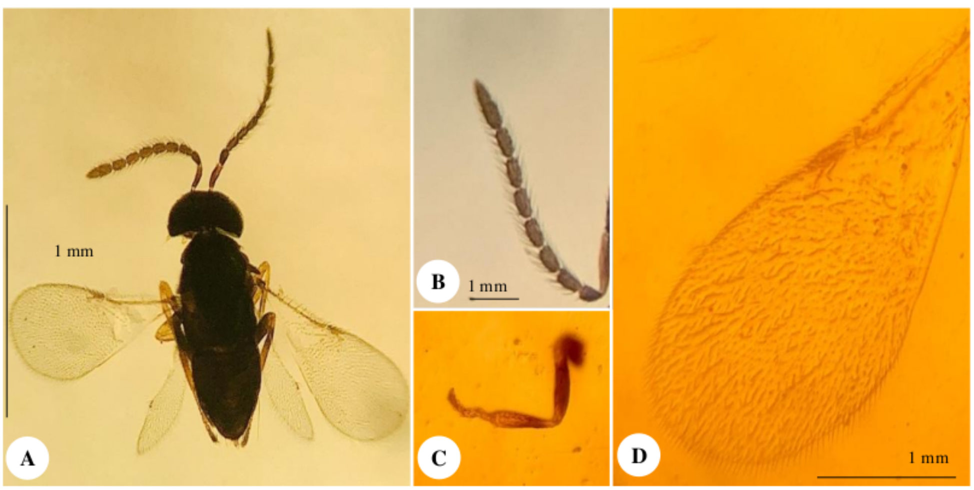

Figure 3. Morphology of *Microterys angustus*: A. full body, B. antena, C. leg, D. wing

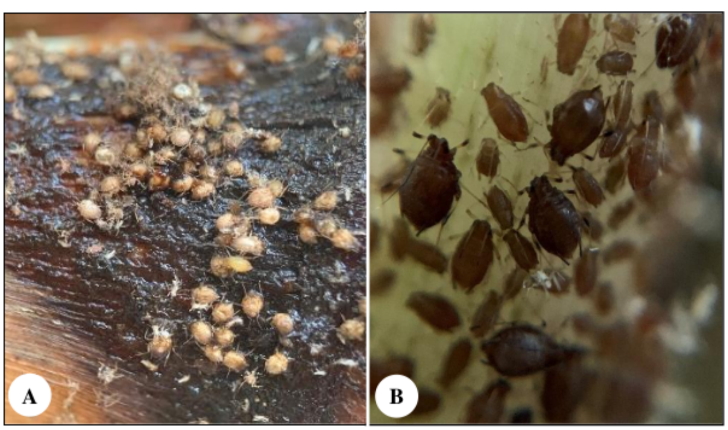

Figure 4. Colony of *Pentalonia nigronervosa*: A. Mummified colony, B. Healthy colony

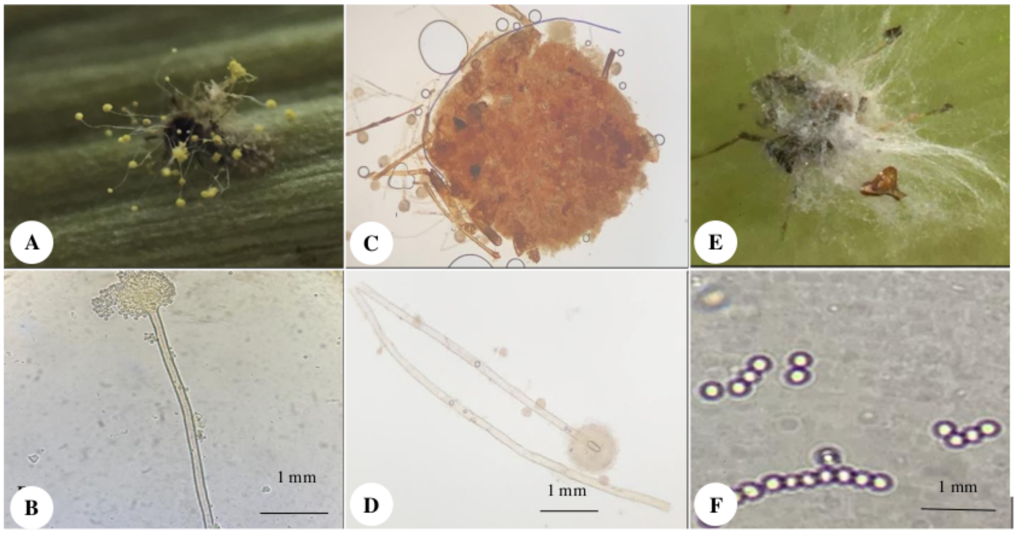

Figure 5. Entomopathogenic fungi infecting *Pentalonia nigronervosa*: A. aphid body infected by *Aspergillus* sp., (a), B. spores of *Aspergillus* sp., (a), C. aphid body infected by *Aspergillus* sp., (b), D. spores of *Aspergillus* sp. (b), E. aphid body infected by *Beauveria* sp., F. spores of *Beauveria* sp.

In this research, a parasitoid i.e., *Lipolexis bengalensis,* was identified as a natural enemy of *P. nigronervosa*. According to Rezaei et al. (2019), there are two orders of insect where a lot of their members live on other insects as parasitoids i.e., Orders Hymenoptera and Diptera. *L. bengalensis* found parasitizing *P. nigronervosa* had brownish body color with body length of ±1.6 mm. This is in accordance with the report of Kocić et al. (2020), who observed that *L. bengalensis* had dark brown head with yellowish-brown mouthparts. Based on parasitic ability assessment, *L. bengalensis* was found to be able to parasitize a half of given population of *P. nigronervosa*. *Lipolexis oregmae* parasitizes *Aphis craccivora*, *A. gossypii*, *Cerataphis* sp. *Hysteroneura setariae* and *A. citricidus* (Miller 2019). Generally, an aphid parasitoid has ability to parasitize all instar of its host (Perdikis et al. 2004). A third instar of *A. gossypii* parasitized by *Lipolexis oregmae*, was reported to be more parasitoids than other instars of aphid (Prasad and Singh 2020). Lipolexis was a polyphagous predator that preyed on various aphid species, such as *Aphis craccivora, A. gossypii, A. spiraecola, Toxoptera aurantii,* and *T. citricida* (Persad et al. 2004; Parween and Ahmad 2015; Kaliuzhna 2019). *L. bengalensis* parasitizing *P. nigronervosa* was also found to be parasitized by hyper-parasitoid *Microterys angustus* (Hymenoptera: Encrytidae). The hyper-parasitoid could parasitize *L. bengalensis* at a high rate of parasitism and their imagoes emerged from the mummies of *P. nigronervosa*. According to Kumar et al. (2019), there are 14 genera of family *Encrytidae* live on other insects as parasitoids or hyper-parasitoid. One of them was *Microterys*. *Microterys* were also reported to parasitize various species of aphids belonging to Coccidae families and Pseudococcidae (Hansen and Japoshvili, 2013; Chelav et al. 2018). Other species of *Microterys* also known as natural enemies with wide host range, such as *M. bellae, M. cneus, M. darevskii, M. hortulanus, M. masii*, and *M. sylvius* (Ghahari and Abd-Rabou 2012). The condition of *P. nigronervosa* parasitized by *L. bengalensis* without hyper-parasitoid was different from those parasitized by *L. bengalensis* and hyper-parasitized by *M. angustus.* The mummies of *P. nigronervosa* were parasitized by hyper-parasitoid, produced wider hole for the hyper-parasitoid to emerge. Entomopathogenic fungi infecting *P. nigronervosa* were *Beauveria* sp., *Aspergillus* sp.(a), and *Aspergillus* sp.(b). *Beauveria* is a biological control agent that suppresses the aphid population without infecting their natural enemies (Akmal et al. 2013). *Beauveria* spp. could infect aphid and produce toxin or chitinase enzyme (Kim et al. 2018). Usually, *P. nigronervosa* is parasitized at life stage of instar 4, and this may be beneficial to the control of BBTV because, instar 4 has the potential to give birth to *P. nigronervosa* and instar 4 can transmitted BBTV higher than other instars. According to Anhalt and Almeida (2008), highest efficiency of BBTV transmission by *P. nigronervosa* was reached when the vector at their fourth stage of their life. *B. bassiana* has also been used in combination with neem leaves extract to control *Sitobion avenae* (Ali et al. 2018). Other entomopathogenic fungi have also been reported to control the aphid population effectively. Erol et al. (2020) reported that application of *Beauveria bassiana* and *Verticillium alfalfa* could successfully control *Aphis gossypii*. From the result of research, *Aspergillus* sp. (a&b) which were found attack *P. nigronervosa* growing and attached to the body of *P. nigronervosa* with an orange-white color, spores attached firmly and not brittle like secondary pathogens. Controlling *P. nigronervosa* by using natural enemies in combination with intercropping banana and tomato has been reported to be more effective (Lifake et al. 2018). The use of pesticides to control *P. nigronervosa* is not commonly suggested because pesticides could only be effective against *P. nigronervosa* on unfolded leaves but not against aphids present in cigar leaves (Robson et al. 2007b). Banana infected by BBTV is usually infested by a crowd of *P. nigronervosa*, and aphid tent to move to young suckers, which are normally used as transplanting material (El-dougdoug et al. 2006). This means that young suckers from infected banana mat most probably have contained BBTV particle even though no symptom has occurred. Therefore, it is important to consider the presence of banana aphid when banana suckers are used as planting materials. Since *P. nigronervosa* act as BBTV vector, we need to know when and how *P. nigronervosa* distribution occurs, this may be an attempt to manage the spread of BBTV through *P. nigronervosa*. In West Bengal, it was reported that the colony of *P. nigronervosa* was at its highest in the second week of December (Basak et al. 2015). The influence of geographic factors should also be considered when the control of *P. nigronervosa* is designed to be successful (Foottit et al. 2010).

In the implementation of biological control, Sharma et al. (2016) reported the use and combination of various natural enemies, such as predators, parasitoids, and entomopathogens. In this research, it was observed that predators, parasitoids, and entomopathogens were able to control the population of *P. nigronervosa*. Therefore, all the identified natural enemies can use in a proper combination to control banana aphid. González-Mas et al. (2019) reported that entomopathogenic fungi could be combined with predators and parasitoids in the implementation of integrated pest management, while (Kakati and Nath 2019) reported the recommended control is the use of virus-free plants and pesticides can be applied to reduce vector population and disease incidence in the field.

In conclusion, banana aphid *P. nigronervosa* infesting various banana cultivars in South Sumatra had natural enemies comprised of predators, parasitoids, and entomopathogenic fungi. The use of identified natural enemies to control banana aphid population is quite promising. Since, under the experiment, the predator showed good predation capacity, the parasitoid showed high parasitic ability, and the entomopathogenic fungi also showed high pathogenicity to the aphid.

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