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Population dynamics of the brown planthopper (*Nilaparvata lugens* Stål.) in rice fields and their association with the entomopthoralean fungus

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Abstract. The rice plant (*Oryza sativa* L.) is one of the primary food sources for Indonesian people. The Brown planthopper (BPH), *Nilaparvata lugens* is one of the most devastating rice insect pests in Indonesia. The entomopthoralean fungus, *Pandora delphacis* is reported to be able to infect BPH naturally in the rice field. This research aims to identify the entomopthoralean fungi phase and determine the level of fungal infection and its relations with the population dynamic of BPH in rice fields. This research was conducted in 2015 and 2016 on rice fields at Bogor Regency and Subang Regency; and in 2017 at Karawang Regency, West Java. The sampled BPH were collected from rice fields. The microscope slide squash mounts in lactophenol cotton blue were made for the insects and it was examined with a microscope compound to determine if hyphal bodies, conidiophore, primary conidia, secondary conidia, saprophytic fungi, and resting spores were present. The results showed that the entomopthoralean fungi phase found on BPH are hyphal bodies, primary conidia, secondary conidia, and saprophytic fungi. The fungus infected all the phases of the BPH, from the first nymphal phase to adult.

Keywords: Biological control, conidia, hopperburn, nymph, food safety

1. Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Hemiptera: Delphacidae), is considered as one of the major rice insect pests in Indonesia. The outbreak of this pest occurred in 2010 and 2011, attacking rice plantations of almost 137,3768 ha and 218,060 ha with a yield loss of 1-2 tons/ha [1]. The BPH attacks rice plants by sucking the stem of the plant and using fluid from the phloem. The symptoms of damage of BPH are that the leaves of the rice plant turn yellow and then dry quickly, known as hopperburn [2]. The BPH pest can attack rice plants from the vegetative phase until they enter the generative phase [3]. The rice plants planted asynchronously can cause the BPH to always be present in the field [4].

To control BPH in the field, it can be applied in various ways that are integrated into one holistic system, known as integrated pest management (IPM) [5]. One of the control techniques that is the backbone of the IPM is biological control by using natural enemies of BPH such as predators, parasitoids, and insect pathogens. One of the insect pathogens that can be used for controlling BPH is the entomophthoralean fungus [6]. This fungus is an obligate pathogen that has a specific host and has a great potential as a natural enemy on several insect's pest and mites because this fungus can sporulate and germinate quickly and has epizootic capabilities [7], reducing the host population to near zero on a particular scale [8].

One of the entomphthoralean fungus, *Pandora delphacis* (Hori) Humber was first reported to infect BPH in 1906 with limited hosts from sucking piercing insects such as leafhoppers and aphids [9]. This fungus has potential as a biological control agent for BPH in rice plants [10]. The fungus is widely reported to infect insects from the Delphacidae and Cicadellidae families, especially planthoppers on rice plants [11]. The presence of entomophthoralean fungus in Indonesia has previously been reported to infects insect pest, such as mealybugs on papaya plants [12], several species of mealybugs on ornamental plants [13], and red mites on sweet potato plants [14]. The knowledge of the existence of the entomophthoralean fungus can be used as a refference for the developing biological control agents for insect pests, especially significant insects' pests that attack plants such as BPH.

However, information regarding the entomophthoralean fungus that infects BPH on rice plants at Indonesia is still limited. Therefore, this research is essential to determine the presence the entomophthoralean fungus as the natural enemy of BPH in rice plants in the field. The objective of the reseach is to determine the presence of entomphoralean fungus, *P. delphacis* on the BPH insects, determine the fungus infection level, as well as its relationship with the population dynamics of the BPH, *N. lugens* in rice plantations It is expected that this research can provide initial information regarding the role and use of the entomophthoralean fungus which infects BPH in Indonesia so that it can be used as a refference for biological control of BPH.

2. Methods

The first study was carried out from December 2015 to June 2016 at three locations: Cibitung Kulon (6°39'42"S 106°38'16"E) and Cibitung Wetan Village (6°39'01"S 106°38'09"E), Pamijahan District Bogor Regency; one location at Kiarasari Village (6°25'56"S 107°53'05"E), Compreng District, Subang Regency. This research continued in March-August 2017, at Cikangkung Village (6°08'35"S 107°18'03"E), Rengasdengklok District, Karawang Regency, West Java. The identification of the phases of the entomopthoralean fungus was carried out at the Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB University (6°33'33"S 106°43'44"E).

2.1. The insect sampling of rice field

To determine the association of BPH with *P. delphacis*, BPHs were sampled in two locations at Bogor Regency and one location at Subang Regency. This research aims in 2015 and 2016 were to determine whether entomophthoralean fungus infect BPH, identify the fungus phase, and determine the fungus infection level on BPH in rice plantation. Sampled BPH in this study were not differentiated based on insect instar. The ten rice plants were randomly determined as sampled plants for each observation location. The Numbers of ±50 BPHs were sampled weekly at each observation site for three times. The sampled BPH were put into a 30 ml bottle filled with 70% alcohol and taken to laboratory for futher examination.

The aim of research carried out in 2017 at Karawang was to determine the relationship between BPH population dynamics and fungal infections. The phases of sampled BPH were differentiated based on the adult forms macropterous or brachypterous adults, and immature nymphal insects.

BPH sampling was carried out on rice plants at Karawang Regency with a land area of 1,000 m^2. The sampled plot was divided into five sampled subplots with an area of 200 m^2/subplot. The twenty-five plants were sampled diagonally for each subplot. A Total of ±100 BPH was sampled weekly at each sampling plot, from rice plants aged six weeks after planting (WAP) to 12 WAP. The sampled BPH from each observation location were put into the jar containing 70% alcohol for the following observation. Number of sampled BPH each plot and each location were determined by:

$$\text{Number of BPH} = \frac{\text{Number of BPH found}}{\text{number of sample}} \quad (1)$$

2.2. Identification of entomophoralean fungus, P. delphacis

The BPH specimens of relatively the same size are arranged on a glass object. The microscope slide squash mounts in lactophenol cotton blue were made for BPH. Edge of the cover glass is smeared with transparent nail polish as an adhesive. The slide preparations are labelled with the location and date of sample collection. The slide preparations were observed under a light microscope with a magnification of 400x. The sampled BPHs was classified into one of the following six categories based on [15]: Healthy insects, BPH infected by secondary conidia, BPH infected by the hyphal body, BPH infected by primary conidia, BPH infected by resting spores, BPH infected by secondary fungi which associated with *P. delphacis*. Infection levels of *P. delphacis* on BPH are determined by

$$\text{Infection level of } P.\ delphacis\ (\%) = \frac{\sum \text{infected BPH}}{\sum \text{observed BPH}} \times 100\% \quad (2)$$

2.3. Data analysis
Numbers of BPH, the proportion of BPH-infected *P. delphacis*, and fungus infection levels were tabulated and determined by Microsoft Excel 2013.

3. Results

3.1. Association between BPH and P. delpachis
Research results in 2015 and 2016 showed that numbers of BPH infected by the entomophthoralean fungus, *P. delpachis* occurred in rice plantations at Bogor and Subang Districts. The phases of *P. delpachis* found were secondary conidia, hyphal body, primary conidia, and secondary fungi, which are associated with *P. delphacis.* The healthy insects are characterized by intact bodies and a bright-looking abdomen (Fig. 1a). In contrast, BPH infected by the fungus have blackened and damaged bodies (Fig. 1b). Secondary conidia are round to ovoid with papillae at the tip were found attached to antennae, legs, or other part body of BPH (Fig 1c-d). These structures are found outside the host body or at the tip of conidiophores. Hyphal body is round and is found to fill the buccal cavity of the BPH (Fig. 1e-f). The primary conidia are round to ovoid in shape (Fig. 1g-h). Meanwhile, secondary fungi were found in the bodies of BPHs that died from being infected with the entomophthoralean fungus, *P. delpachis*. Unlike entomophthoralean fungus, secondary fungi have protoplasm in their hyphae or spores interrupted at regular intervals by partitions septa (Fig. 1i).

The most critical criteria in identifying entomophthoralean fungi are based on the host and the characteristics of its structures [16]. The primary conidia found were round to ovoid in shape with a papilla at the tip and the secondary conidia were oval in shape. These results are following with [17] those primary conidia are clavate to ovate with rounded papilla, and secondary conidia resemble primary conidia or rounded conidia, which are characteristics of the genus *Pandora*. Meanwhile, the form of secondary conidia secondary conidia found was different from the primary conidia and included type 1b. Based on these criteria, the entomophthoralean fungus that infects BPH at the observation sites was identified as *P. delphacis*. The fungus, *P. delphacis* was found to infect 1 species from the order Lepidoptera and seven species from the order Hemiptera, namely four species from the Delphacid including BPH, three species from the Cicadellid, and one species from the Membracid [18].

The secondary conidia were found to infect BPH at the observation site, in the third observation in Bogor and were found in the second and third observation in Subang (Table 1). The secondary conidia are the infectious phase of the entomophthoralean fungus. This phase can infect healthy BPH in rice fields. The secondary conidia first attach to BPH body parts such as antennae, legs, or other body parts. After that, under optimal environmental conditions, these structures will germinate and penetrate the insect body.

The hyphal bodies were the most found in BPH at all observation sites. Presence of hyphal bodies continues to increase in Cibitung Wetan, Bogor, while in Kiarasari, Subang decreased, and in Cibitung Wetan, it fluctuated (Table 2). The hyphal body phase is the next after the secondary conidia have successfully penetrated the BPH body.

Primary conidia are the next phase formed from the tip of the conidiophore, which penetrates outside the BPH body. Primary conidia will be sporulated and spread around the BPH cadaver and, under optimum environmental conditions can form a capillary structure at the end of secondary conidia, or capilloconidia are formed.

BPHs infected with the primary conidial phase were found in all three observation sites with a high proportion. The Table 1 shows the high proportion of BPH infected by primary conidia followed by a decreasing proportion of BPH infected by hyphal bodies and vice versa. Secondary fungal structures were also found in BPH cadavers that died due to entomophthoralean fungus infection. These structures were not found very often because it is not part of the entomophthoralean fungus phase.

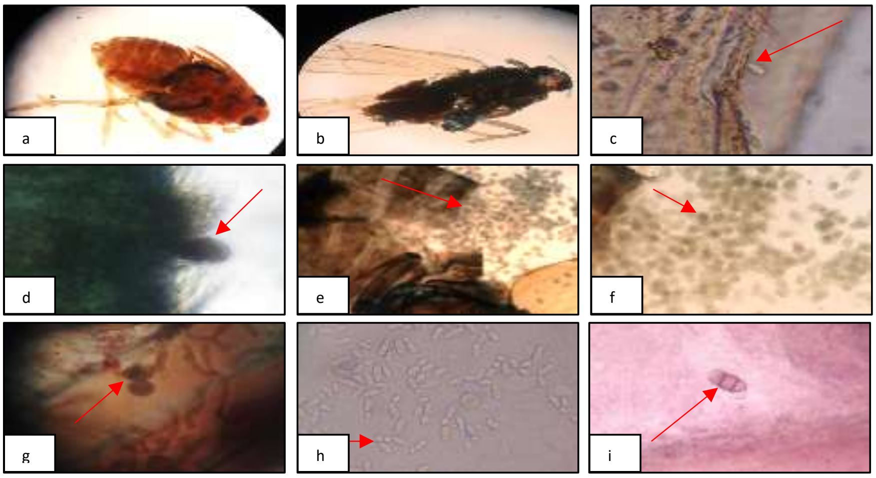

Figure 1. Structures of entomophthoralean fungus, *P. delphacis* (a) Healthy BPH, (b) infected BPH, (c-d) secondary conidia, (e-f) BPH infected hyphal bodies, (g-h) BPH infected by primary conidia, (i) secondary fungi which associated with P. *delphacis*

3.2. Population dynamics of BPH on rice field

During observation in 2017, BPH pests were found from the beginning of observation on rice at 6 WAP (a week after planting) until the last observation at 12 WAP. The BPH can infest rice plants from the vegetative to generative phases [11]. The number of BPH at 6 WAP and 7 WAP was 2.64 and 3.36 BPH/plant, respectively (Fig. 2). The most common BPH phase found at 1^{st} two observation times was brachypterous adults (Table 2). The same results were described by [15] that BPH adults that appear on rice plants after entering 7 WAP are generally short-winged adults which will lay their eggs on surrounding rice plants. The influence of rice stems that have grown tall at 6 and 7 WAP is thought to be a factor in the large population of brachypteran adults for their lives and reproduction. The eggs laid hatch and become nymphs on following observation. Number of nymphs at 8 WAP increased drastically to 100%. That is no other BPH phases were found apart from the nymph phase (Table 2). The number of BPH at 8 WAP reached 15.8 BPH/rice plant, and it was the highest number during observation.

The BPH nymphs have the most potential to damage rice plants [5]. This because the nymphs need a lot of food and nutrients to develop, so the large number of feeding activities carried out by the nymph phase can cause damage to rice plants. The high population of the BPH nymph phase occurred three times, at 8, 10, and 12 WAP. The absence of the nymph phase in several WAPs of rice plants was replaced by the appearance of brachypteran and macropteran adults (Table 2).

The BPH population dynamics show that the population continues to increase when the rice plants are in the early vegetative and generative phases and then continues to decline when the rice plants enter the mature stage. This can be influenced by nutritional substances contained in rice plants. In the vegetative phase, rice plants contain high levels of nitrogen, which is the main living requirement for BPH, while in the generative phase, rice plants tend to need phosphorus and potassium for the formation of rice grains. The low nitrogen in mature rice plants stimulates BPH to form macropteran adults to find a more suitable host [13]. Meanwhile, the result shows that in the last observation at 12 WAP, only BPH nymphs were found even though the population was decreasing (Table 2).

Table 1 Proportion of BPH infected by entomophthoralean phase on rice plant at Bogor and Subang in 2015-2016

BPH infected by	Proportion of infected BPH (%)								
	Cibitung Wetan (Bogor)			Cibitung Kulon (Bogor)			Kiarasari (Subang)		
	n=11	n=16	n=19	n=32	n=69	n=34	n=15	n=47	n=143
Non-HeB	18.18	18.75	5.26	12.50	4.35	2.94	0.00	0.00	0.00
SC	0.00	0.00	10.53	0.00	0.00	2.94	0.00	8.51	1.39
HB	27.27	37.50	78.95	62.50	85.51	52.94	93.33	59.57	42.67
PC	54.55	43.75	5.26	25.00	8.69	41.18	6.67	29.79	55.94
RS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SF	0.00	0.00	5.88	0.00	1.45	0.00	0.00	2.13	0.00
IL	81.82	81.25	94.74	87.50	95.65	97.06	100	100	100

n = Numbers of sampled BPH, Non-HeB=Healthy BPH or non infected BPH, SC= Secondary conidia, HB=Hyphal bodies, PC=Primary conidia, RS= Resting spore, SF=Secondary fungi, IL=Infection level

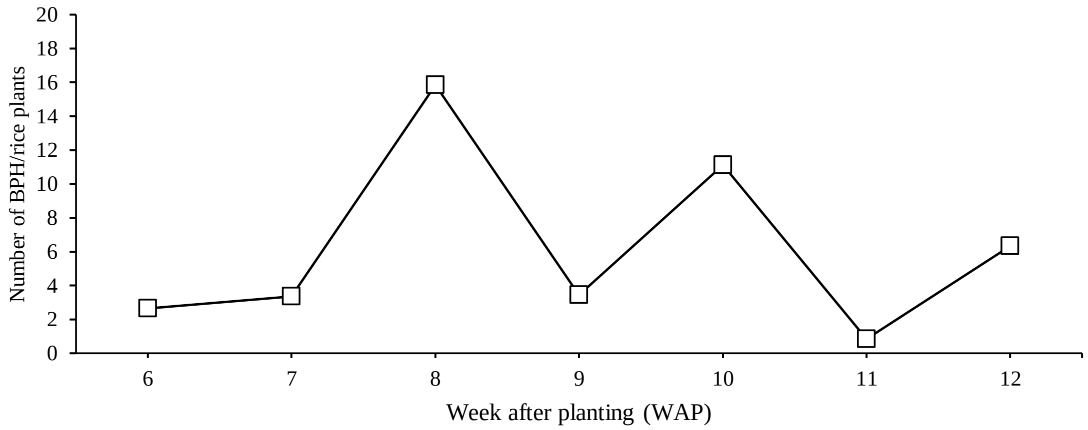

Figure 2 Population dynamics of *N. lugens* (BPH) at various stage of rice plants (number of sampled BPH/rice plant) at Karawang in 2017

3.3. Infection level of P. delphacis on BPH at Karawang

The entomophthoralean fungus, *P. delphacis* was also found to infect BPH on rice plants at Karawang. Table 3 shows that the infection level of this fungus fluctuates, with the highest infection level occurring in third week of observation, reaching 93%, and in the sixth week of observation, reaching 81%. The proportion of BPH infected by fungal phases was dominated by hyphal body phases. The secondary and primary conidia were also found to infect BPH in a smaller proportion. The entomophthoralean fungus has a high potential to suppress BPH populations. On Table 3 also shows that this fungus continues to suppress the healthy BPH population, reaching 50% of the population, and even more than 90% of infected BPH occurred at rice plants.

Table 2. Number of BPH phase per rice plant on various rice plant phases (individual/rice plant) at Karawang in 2017

Sampling date (WAP)	Number of sampled BPH phase (individual/ rice plant) and percentage (%)		
	Nymph	Brachipterous adults	Macropterous adults
6 (n=66)*	3 (4.5%)**	59 (89.4%)	4 (6.1%)
7 (n=84)	0(0%)	72 (85,7%)	12 (14,3%)
8 (n=396)	396 (100%)	0 (0%)	0 (0 %)
9 (n=86)	0 (0%)	48 (55,8%)	38 (44,2%)
10 (n=278)	262 (94,2%)	16 (5,8%)	0 (0%)
11 (n=13)	0 (0%)	12 (57,1%)	9 (42,9%)
12 (n=158)	158 (100%)	0 (0%)	0 (0%)

*n = Total number of sampled BPH, ** number outside parentheses= individual number and number inside parentheses=proportion of sampled BPH

Table 3. The proportion of infected BPH by entomophthoralean fungus, *P. delphacis* on a rice field at Karawang in 2017 (%)

Sampling date (WAP)	Healthy BPH	BPH with SC	BPH with HB	BPH with PC	BPH with RS	BPH with SF
6	70.0	0.0	30.0	0.0	0.0	0.0
7	62.0	2.3	35.7	0.0	0.0	0.0
8	7.0	0.0	93.0	0.0	0.0	0.0
9	45.0	0.0	54.0	1.2	0.0	0.0
10	51.0	0.0	49.0	0.0	0.0	0.0
11	19.0	0.0	81.0	0.0	0.0	0.0
12	55.0	1.3	44	0.0	0.0	0.0

WAP= week after planting, SC= Secondary conidia, HB=Hyphal bodies, PC=Primary conidia, RS= Resting spore, SF=Secondary fungi

The fungus was found to infect all BPH phases (Fig. 3). Fungus infection occurred during the first observation (6 WAP) on brachypteran and macropteran adults. A highest infection level occurred at 8 WAP at 93%, with several BPH infected as many as 368 individuals. All BPHs fungus-infected found were in the nymphal phase. The more vulnerable body characteristics of BPH nymphs and their high population make it possible for the fungus to infect many hosts. The active foraging behavior of BPH nymphs allows much contact between healthy BPH and infected BPH to help the fungus spread.

3.4. Association fungus infection levels with the population dynamics of BPH

The entomophthoralean fungus is the defendant factor. An increase in the BPH population is followed by an increasing infection level of entomophthoralean fungus, while a low population remains followed by an increasing fungus infection (Figure 4). This can be influenced by characteristics of the entomophthoralean fungus, which is obligate, so it continues to need a living host for its development and spread.

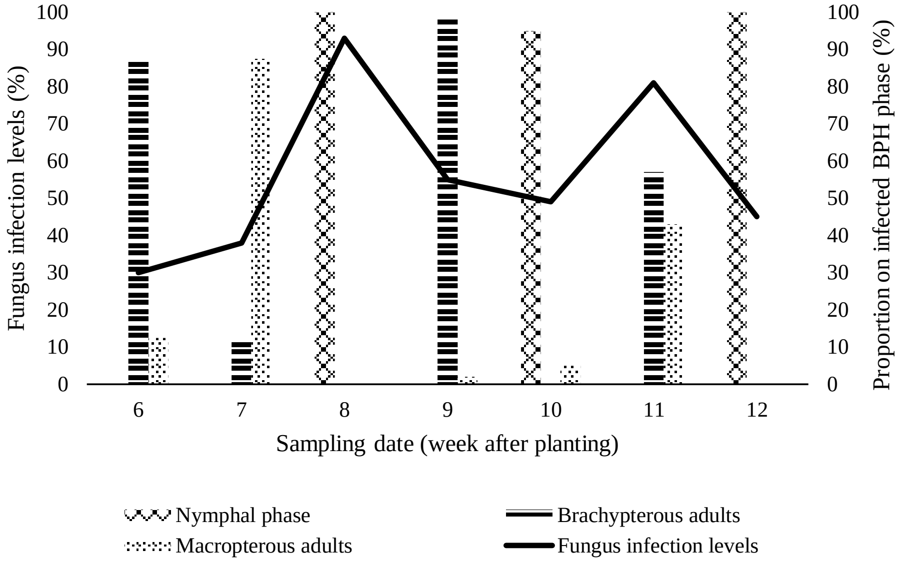

Figure 3. Fungus infection levels and the proportion of infected BPH in rice field at Karawang in 2017

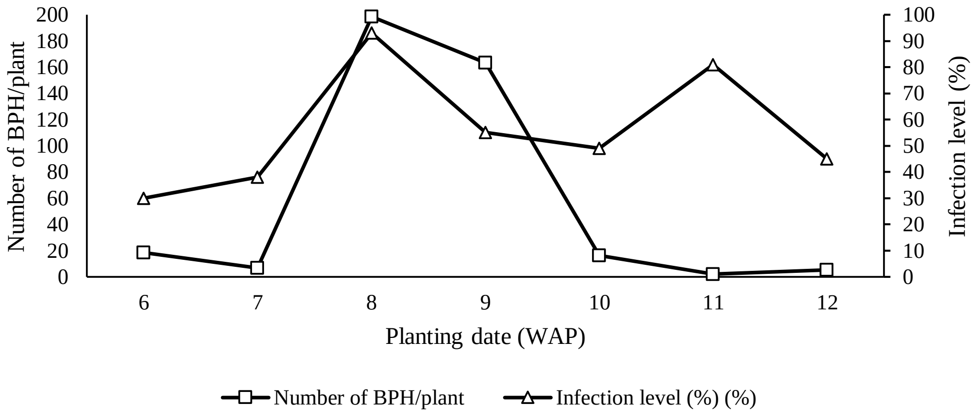

Figure 4. Association between fungus infection, *P. delphacis*, with population dynamics of BPH on rice plants at karawang in 2017

4. Discussion

BPH, *N. lugens* was initially a secondary pest. However, with changes in plant cultivation, including the use of superior varieties that have more tillers and close plant spacing, the microclimate has become more suitable for the development of pests. Unwise use of insecticides also causes problems of pest resistance and resurgence, death of non-target organisms. Over the last few decades, the BPH has increased from an infrequently occurring pest to a major pest of rice in several tropical regions in Asia [19].

The recommended control of BPH in the field is through integrated pest management (IPM). This IPM strategy combines various control techniques that are economically profitable, ecologically sustainable, and socially acceptable to farmers. One of the backbones of IPM is biological control by using natural enemies of pests, such as predators and parasitoids, and pathogens. These three types of natural enemies are generally available in nature. Our job is to utilize or optimize their role in nature. One type of pathogen that can be utilized is the entomophthoralean fungus, most of whose hosts are insects. Unfortunately, although this fungus is often found in nature and has an important role in controlling pests, it is relatively difficult to reproduce in the laboratory. Thus, it is our duty to ensure the conservation of natural enemies runs well in nature. This activity is carried out to improve the life and safety of natural enemies in nature. Let the fungus live and let them work. Several cultivation techniques must be carried out to ensure the life of these natural enemies

One of the entomophthoralean fungi reported to infect BPH is *Pandora delphacis*. Our research conclude that this fungus was found to infect BPH in rice plantations at Bogor, Subang and Karawang Regencies. We found four of five phases of this fungus to infect BPH, namely secondary conidia, hyphal bodies, primaru conidia, and secondary fungi. The hyphal bodies form and reproduce in hemocoel [20]. This fungus structure plays a role in taking nutrients until the host insect dies [21]. The BPH bodies filled with hyphal bodies are generally blackened and damaged (Fig 1b). The structure of the hyphal bodies continues to multiply to fill the contents of the insect body and then form conidiophores, which will penetrate outward insect body [19]. Large number of hyphal body structures in infected BPH because these structures are formed after secondary conidia and before primary conidia, and their role is in paralyzing and killing the host insect within a few days. Therefore, BPH that has been infected and died due to the entomophthoralean fungus allows hyphal bodies to be found in the insect body. These structures can be found directly with other structures of the fungus.

During the study, resting spores were not found to infect BPH. It is understood that BPH as a host is always present in the field. In addition, the weather is always suitable for developing of the fungus. The formation of these spores is usually triggered by the absence of a host and extreme weather conditions. In subtropical countries, the formation of resting spores always occurs because both insects and their natural enemies must diapause during the winter.

In our research, we also found that all stages of BPH were infected by this fungus, both nymphs, adult macroptera and adult brachiptera. There is a positive correlation, a high BPH population will be followed by fungal infections in BPH which are also high.

5. Conclusion

Three peak populations of BPH occurred during this study, with the highest population level at 8 WAP and was dominated by nymphal phases. The phases of *P. delphacis* discovered in 2015, 2016, and 2017 were hyphal body, primary conidia, secondary conidia, and secondary fungus. Entomophthoralean fungus found on BPH was identified as *Pandora delphacis*. This fungus infects all BPH phases, from the first nymph to the adult. The fungal infection level and BPH population had a positive correlation. The presence of the entomophthoralean fungus, *P. delphacis* in the field only occurs in living insects because the fungus is obligate. Therefore, conservation efforts such as the application of pesticides, especially fungicides, are carried out wisely.

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