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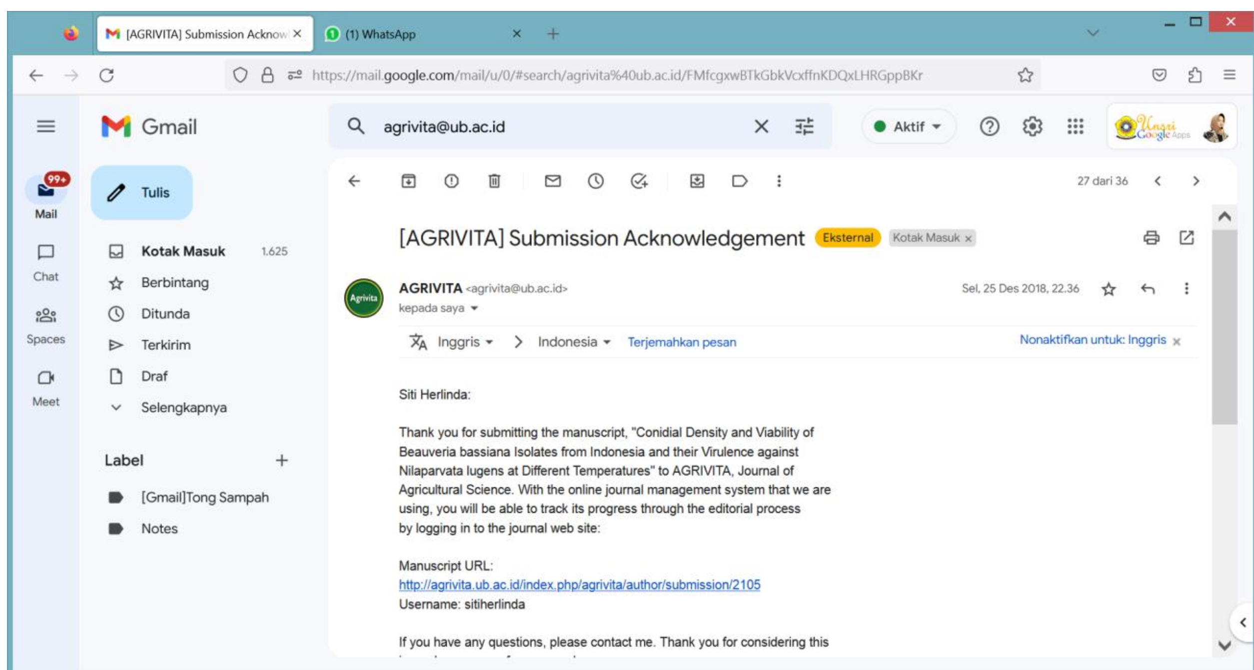

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AGRIVITA <agrivita@ub.ac.id>
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I. Conidial Density and Viability of *Beauveria bassiana* Isolates from Indonesia and their Virulence against *Nilaparvata lugens* at Different Temperatures

Eka Sumikarsih[1], Siti Herlinda[2,3*] and Yulia Pujiastuti[2,3]

II. First author:

1. Name : Eka Sumikarsih
2. Afiliation : [1]Graduate Program of Crop Sciences, Faculty of Agriculture, Universitas Sriwijaya, Palembang, Indonesia 30139

3. E-mail : sumikarsiheka@gmail.com
4. Orcid ID : https://orcid.org/0000-0002-9015-5561
5. Contribution to this Manuscript: focused on preparation of test insects, isolates preparation of *Beauveria bassiana*, observation of conidial density and viability, and bioassays

III. Corresponding author:

1. Name : Siti Herlinda
2. Afiliation : [2]Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, Indonesia 30662
[3]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang, Indonesia 30139
3. E-mail : sitiherlinda@unsri.ac.id
4. Orcid ID : https://orcid.org/0000-0003-1460-7199
5. Contribution to this Manuscript: made the whole research design, managed the whole research, drafted and finalized the manuscript

IV. Third author:

1. Name : Yulia Pujiastuti
2. Afiliation : [2]Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, Indonesia 30662
[3]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang, Indonesia 30139
3. E-mail : ypujiastuti@unsri.ac.id
4. Orcid ID : https://orcid.org/0000-0003-3204-9039
5. Contribution to this Manuscript: focused on conduting data analysis.

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VI. Reviewers Candidate :

1. Purnomo, Orcid ID: https://orcid.org/0000-0001-6132-3936. E-mail: purjomo@yahoo.com
2. Novri Nelly, Orcid ID: https://orcid.org/0000-0003-3830-3763. E-mail: novrinelly@yahoo.com
3. Maoye Li, Scopus ID: 55828064600, E-mail: sj412bq@163.com
4. Jae Su Kim, Scopus ID: 55161782900, E-mail: jskim10@jbnu.ac.kr

Conidial Density and Viability of *Beauveria bassiana* Isolates from Indonesia and their Virulence against *Nilaparvata lugens* at Different Temperatures

ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* can cause direct damage and transmit rice diseases, such as ragged and grassy stunt virus. *Beauveria bassiana* was used to control BPH, however the success of the fungal efficacy on rice fields was affected by external factors, such as temperature. This research aimed to evaluate the conidial viability and density of *B. bassiana* isolates exposed to 25 and 34 °C and their virulence against BPH nymphs. Twenty six isolates of *B. bassiana* cultures incubated at 25 and 34 °C for 7 days were observed their conidial density, viability, and virulence against BPH nymphs. Incubation temperature of 34 °C was able to decrease conidial density and viability, and virulence of the isolates. However, some isolates of *B. bassiana* originated from soils or insects, especially from South Sumatra still produced high conidial density and viability as well as high virulent against BPH nymphs, such as TS1D3A, TSID3B, TS1D2A and TS1D2B isolates. The TS1D2B isolate incubated at 34 °C still caused the highest percentage of BPH mortality (43.33%) among other isolates. Therefore, the isolates can be used as promosing candidate for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as wetland or lowland rice ecosystems in Indonesia.

KEYWORDS

Brown Planthopper, Mortality, LT_{50}, LT_{95}

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae) is the most serious insect pest which sucks phloem sap on stems of rice (*Oryza sativa* L.) (Daravath & Chander, 2017; Herlinda et al., 2018). The direct damage caused by this BPH results in hampered growth of rice which in turn produce of ‘hopperburn’ (Dharshini & Siddegowda, 2015). In addition, BPH can also as an insect vector that transmits rice diseases, such as the ragged stunt and grassy stunt virus (Zheng et al., 2014; Dietzgen et al., 2016). The attack by this BPH not only occured in Indonesia, but also had attacked rice in several Asian countries, such as China, Vietnam, Thailand, India, Pakistan, Malaysia and Philippine (Catindig et al., 2009; Hu et al., 2014). BPH had also attacked rice in Texas (Leavengood et al., 2017).

The effort to control population of *N. lugens* had been done through synthetic chemical control (Liu et al., 2013; Zhang et al., 2014; Baehaki & Suparno, 2018) and biological control by using entomopathogenic fungi, such as *Beauveria bassiana* (Li et al., 2012, 2014; Lee et al., 2015) and *Metarhizium anisopliae* (Chinniah et al., 2016) that had proven to be an effective agents to control the BPH. *B. bassiana* could kill the BPH more than 80% (Li et al., 2014; Lee et al., 2015) and to kill eggs of the BPH as well as to disturb

adult stage proliferation of the BPH (Li et al*.,* 2012). *B. bassiana* is also not harmful toward natural enemies of the BPH (Firouzbakht et al., 2015; Gholamzadeh-Chitgar et al., 2017).

Although *B. bassiana* had proven to be effective in controlling the BPH, the success of its efficacy on rice fields was affected by many external factors, such as temperature (El-Ghany, 2015). Extremely high temperature can results in death of the fungus (Ottati-de-lima et al., 2014). The optimum temperature for growth of the entomopathogenic fungi usually is in the range of 25 to 30ºC (Bugeme et al., 2008). Most of entomopathogenic fungi tolerate to temperatures in the range of 0 to 40°C (El-Ghany, 2015), but certain strains of entomopathogenic fungi can only survive at temperatures below 35 °C (Constanski et al., 2011). Production of colony and conidial density of *B. bassiana* is significantly decrease if temperature during fungal incubation is increased from 30 to 35 °C (Ottati-de-lima et al., 2014) and all isolates are death at temperature of 36 °C (Pham et al., 2009; Ottati-de-lima et al., 2014). Fungal germination is also decrease at temperature above 30 °C (Pham et al., 2009) with the highest level up to 33 °C (Salim et al., 2015), whereas temperature of 25°C is an ideal temperature for the fungal germination (Lohse et al., 2014). Virulence of the entomopathogenic fungi can be affected by temperature (Tefera & Pringle, 2003; Bugeme et al., 2008; Constanski et al., 2011; Ottati-de-lima et al., 2014; El-Ghany, 2015; Satpathi et al., 2016). Each strain/isolate or species of the entomopathogenic fungi also has different optimum temperature and the tolerance level to temperature. Entomopathogenic fungal strains that could survive at extremely high temperature of above 33 °C are superior strains (Salim et al., 2015). These superior strains can be used as candidates to control the BPH in tropical ecosystems, such as agroecosystems in Indonesia. Therefore, the objectives of this research were to evaluate the conidial viability and density of *B. bassiana* isolates exposed to 25 and 34 °C temperatures and their virulence against *N. lugens* nymphs.

MATERIALS AND METHODS

Study Site

This research was conducted at Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, from September 2017 to April 2018. Isolates used in this research were isolates collected from soil of lowland swamps, tidal lowlands, peatlands, and highlands in South Sumatra, whereas isolates from soil and infected insects obtaining from other provinces were used as comparison as well as one commercial isolate was used as control (Table 1). The species of the fungus was identified by Dr. Suwandi, a microbiologist from Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya. Average room temperature during experiment was 30.50 °C and average relative humidity was 84.30% within the laboratory during the experiment.

Preparation of Test Insects

Adults and nymphs of *N. lugens* were collected from fields of rice at Indralaya, South Sumatra since September 2017 up to March 2018 and brought to Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya for identification. Then, the nymph and adults were reared and maintained on 5 clumps of 10-day-old rice grown within bucket (bottom diameter of

20 cm, upper diameter of 25 cm, and height of 20 cm) within a greenhouse at temperature range of 30 to 35 °C. The rice was put in wire mesh enclosure (height of 100 cm, length of 50 cm, and width of 50 cm). Within one of wire mesh enclosure, 10 pairs of the adults were released in order to infect the rice for 10 days and subsequently the infected rice crops were substituted with the healthy one and this propagation was done on 30 cages. Fresh and healthy rice were given to newly emerged nymphs of *N. lugens* and this was maintained up to at least of five generations. The sixth generation and henceforth generations were used and selected for the fourth instar for bioassay in this experiment.

Isolates Preparation of *Beauveria bassiana*

All isolates used in this study were previously fitted by using living insects of *Tenebrio molitor.* Fresh cultures of *B. bassiana* were started by inoculating Sabouraud Dextrose Agar (SDA) (Oxoid) with the third larvae of *T. molitor*. Prior to inoculation, *B. bassiana* and *T. molitor* were previously sterilized by using modified method from Nuraini et al. (2017). Subsequently, the fungal culture was incubated for 7 x 24 hours in order to produce sufficients numbers of fungal colony. The *B. bassiana* culture that had previously fitted was then used for subsequent observation. The fresh *B. bassiana* culture was cut with dimension of 10 mm x 10 mm for recultured into SDA medium which would be used for subsequent observation consisting of conidial density and viability as well as virulence test.

Observation of Conidial Density and Viability

Twenty six isolates of the *B. bassiana* culture were grown on SDA medium and then each isolate was incubated at constant temperature of 25 and 34 °C within incubator for 7 x 24 hours using three replications. The ideal temperature of 25 °C and extremely high temperature of 34 °C in this research were chosen for culturing of *B. bassiana* for 7 days. Both temperature were chosen because temperature of 25 °C is ideal temperature for culture *B. bassiana* (Bugeme *et al.*, 2008), whereas temperature higher than 33 °C is extremely high temperature (Salim et al., 2015). Conidia of all isolates at the 8th day were counted in term of their density of the 7 day *B. bassiana* culture. Calculation of conidial density was started with fungal suspension production by harvesting 10 mm x 10 mm (1 cm^2) 7-day *B. bassiana* culture which followed by 10 mL addition of sterile distilled water. The suspension was vortexed using turbo mixer for 20 seconds in order to produce homogenous conidial suspension. This suspension culture was diluted through addition of 9 mL sterile distilled water into 1 mL *B. bassiana* suspension culture, homogenized. Subsequently, the last suspension culture was counted in term of its conidial density under a compound microscope at 400x magnification that had been equipped with haemocytometer. This treatments were arranged in completely randomized design and replicated three times.

Calculation of conidial viability was based on percentage of conidial germination. The conidial germination was observed from the 7-day *B. bassiana* culture which incubated at 25 or 34 °C and grown on one layer of SDA medium according to the method of Bugeme et al. (2008). The 7-day *B. bassiana* suspension culture was scratched with magnitude of 0.1 mL on SDA plates. A sterile microscope cover slip was placed on each plate, then each suspension culture was incubated for 24 and 48 hours at temperature of 25 °C. Furthermore, the germinated conidia was observed under microscope and calculation was done in term of numbers of germinated conidia. The germination percentage was determined from 100-spores for

each plate using the compound microscope. These treatments were arranged in completely randomized design and replicated three times.

Bioassay Procedure

Bioassays by modification of Herlinda (2010) and Trizelia and Nurdin (2010) method were conducted to determine the virulence of *B. bassiana* isolates againts *N. lugens* nymphs. The 26 isolates of *B. bassiana* were incubated at 25 and 34 °C for 7 days and their conidia were harvested. Each isolates of *B. bassiana* was topically sprayed with 10 ml of a concentration of 1 x 10^3 conidia cm^{-2} on 25 fourth-nymphs of *N. lugens* placed on a filter paper in petri dishes. Then, the nymphs of *N. lugens* were placed into 20 healthy two-weeks rice stems. This treatments were arranged in completely randomized design and replicated three times. Numbers of dead *N. lugens* nymphs was recorded every 12 hours for 11 days period which used to determine the percentage of mortality and lethal time to 50% (LT_{50}) and 95% (LT_{95}) mortality of *N. lugens* nymphs. The behaviour change of *N. lugens* infested by *B. bassiana* was observed and recorded daily until the insects were death and mycelia cover all of their bodies. The dead nymphs were transferred into petri dishes lined with moist-sterile filter paper to allow the growth of the *B. bassiana* on the surface of the cadaver.

Data Analysis

Data of conidial density and viability, and percentage of mortality among treatments were analyzed by using analysis of variance (ANOVA). If there were differences among the data of each treatment, then Honestly Significant Different (HSD) test at 5% was conducted by using program software of SAS University Edition 2.7 9.4 M5. LT_{50} and LT_{95} values were calculated by using probit analysis.

RESULTS AND DISCUSSION

Conidial Density and Viability of *Beauveria bassiana*

B. bassiana culture incubated for 7 days at temperature of 25 °C showed that the highest conidial density was found on BTmSo isolate and was not significantly different than that of BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B isolates (Table 2). At incubation temperature of 34 °C, the highest value of conidial density was found on TS1D2B isolate and was significantly different than that of BPcPd2 isolate which had the lowest conidial density value. Conidial density of all isolates at 25 °C were siginificantly higher than that of the isolates at 34 °C ($P = 0.00$) (Fig. 1). Conidial density significantly decreased with the increase of the fungal incubation temperature and followed by more hampered of fungal colony growth. Fungal culture incubated for 7 days at 25 °C had normal growth with colony diameter in the range of 50 to 90 mm, whereas colony of *B. bassiana* incubated at 34 °C only had diameter in the range of 15 to 30 mm (Fig. 2). Incubation temperature of 34 °C for *B. bassiana* culture was able to decrease conidial density and colony growth of the fungus; however some isolates (TS1D3A, TSID3B, TS1D2A and TS1D2B isolates) were still achieved high conidial density and colony growth.

Conidial density values of *B. bassiana* isolate cultures incubated at 25 °C were all high, but higher values were found on isolates of BtmSo, BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B. They were

isolates from South Sumatra. Conidial density of all isolates were siginificantly decrease if the isolates were incubated at 34 °C. However, only TS1D2B isolate still had high conidial density at incubation temperature of 34 °C. The conidial density was decrease at 34 °C due to lower production of conidia cm^{-2} in agar medium which indicated by hampered colony growth of *B. bassiana* (Fig. 2). The diameter of colony growth reach 90 mm at 25 °C, whereas diameter of colony growth at 34 °C was only 15-30 mm. Ottati-de-lima et al. (2014) had stated that *M. anisopliae* could yield optimal colonies in liquid medium from 25 to 30 °C and temperature of 35 °C was detrimental to colony growth of the fungus. High spore or conidial production of *B. bassiana* was occurred at 25-27 °C (Pham et al., 2009). Incubation temperature of 34 °C in this research could decrease the conidial density and colony growth of *B. bassiana*.

Viable or germinate conidia was characterized by the following aspects: the change of size and form of conidia compared to size and form of normal conidia (Fig. 3a), conidial wall was broken and produce germ tube and followed by elongation of the germ tube (Fig. 3b and 3c). Percentage of conidial germination was used to determine conidial viability. At incubation temperature of 25 °C, the highest value of conidial viability of 24-hour-suspension culture was found on TS1D3A isolate and was not significantly different than that of other isolates, except BTmKt and BLePd isolates (Table 3). However, the highest value of conidial viability at temperature of 34 °C was found on Natural BVR# isolate in control of commercial product and was not significantly different than that of other five isolates consisting of BTmPc, BBY, BtmPe, BTmPd and BTmkbc. Conidial viability of 48-hour-suspension culture at 25 °C for all isolates were high and was not significantly different among isolates. Nevertheless, isolates that had the highest conidial viability at 34 °C was Natural BVR# isolate and was not significantly different than that of BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B isolates. Temperature increase during incubation of *B. bassiana* had significant effect on conidial viability (Fig. 4). Conidial viability was significantly decrease if the *B. bassiana* culture was incubated at 34 °C, either for 24-hour-suspension culture ($P = 0.00$) or 48-hour-suspension culture ($P = 0.00$). Therefore, incubation temperature at 34 °C for 7 days for *B. bassiana* culture was capable to decrease the fungal conidial germination, although some local isolates of BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B could produce high percentage of germination.

The shape of conidial germination of *B. bassiana* in this research was similar to description of conidial germination given by the following researcher. Conidial germination showed the following signs: broken conidial wall resulting in germ tube formation which had long stretch and increase in size or diameter of conidia (Guilherme et al., 2015; Safitri et al., 2018). Percentage of conidial germination was used to measure conidial viability of the fungus. Conidial viability of *B. bassiana* at 25 °C was high on TS1D3A, BTmKt and BLePd isolates. However, the conidial viability that was still high was found on TS1D3A, commercial BVR#, BTmPc, BBY, BTmPd, TSID3B and TS1D2B isolates at 34ºC. The conidial viability of *B. bassiana* in 24-hour suspension or 48-hour suspension culture was decrease significantly at incubation temperature of 34 °C. Ideal temperature for the conidial germination of *B. bassiana* was in the range of 25 to 27 °C, but *B. bassiana* conidia could still germinate at 32 °C (Constanski et al., 2011). Salim et al. (2015) had stated that the conidial germination of *B. bassiana* was still occurred up to 33 °C. Local isolates of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B originated from soils and insects of South Sumatra and East Java, Indonesia were still had high conidial viability at 34 °C. The conidial viability of these local isolates were equivalent to those of the commercial BVR# isolate. The conidial isolates that were capable to

germinate at 34 °C were rarely occurred, but only high temperature resistant isolates that had capability to germinate. According to Salim et al. (2015) only superior strains of the fungi were able to germinate at above 33ºC. The conidia of the local isolates in this experiment that were capable to germinate at 34ºC had potential to be developed as active ingredients of bioinsecticides to control *N. lugens* in high temperature rice ecosystem, such as wetland or lowland rice ecosystems of South Sumatra, Indonesia. According to Lakitan et al. (2018a,b) temperature occured in wetland or lowland rice ecosystems of South Sumatra was above 30 °C.

Virulence of *Beauveria bassiana*

The symptom of *N. lugens* nymphs infected by *B. bassiana* was started to appear at second day after being exposed to the fungal conidia with doses of $1x10^3$ conidia cm^{-2}. The nymphs had slow movement and finally stop moving, whereas the healthy nymphs still actively moving at rice stem. On the third day, some of the sick nymphs were death, whereas the other infected nymphs which were still alive became unable to move anymore with their legs and stylets attached on rice stem. On the fourth and fifth days, the dead nymphs hung with their stylets still attached on rice stem, whereas all their legs were not grip on rice stem anymore. On the sixth day, the dead nymphs became hardened and stiff. On the seventh day, their bodies wrinkle, decay with no smell and their integuments were coated with white color mycelia which gradually become brownish white to dark brown colors (Fig. 5).

Virulence of *B. bassiana* isolates against *N. lugens* nymphs was represented on percentage of mortality and the lethal time to 50% (LT_{50}) and 95% (LT_{95}) mortality of *N. lugens* nymphs caused by *B. bassiana.* All isolates of *B. bassiana* tested in this experiment were pathogenic against the *N. lugens* nymphs. At temperature of 25 °C, mean mortality of *N. lugens* nymphs with magnitude of 96.67% was found on TS1D2B isolate, whereas the lowest mean mortality with magnitude of 20% was found on BPcPd2 isolate (Table 4). The highest value of mean mortality of *N. lugens* nymphs at 34 °C was found on TS1D2B isolate (43.33%), wehereas the lowest value of mean mortality of *N. lugens* nymphs was found on Bws Pantura isolate (5%). However, mortality of *N. lugens* nymphs caused by *B. bassiana* was not significantly different among all isolates either at temperarture of 25 or 34 °C. The percentage of *N. lugens* mortality was significantly decrease if fungal incubation temperature was increased from 25 to 34 °C (P = 0.03) (Fig. 6). Therefore, incubation temperature at 34 °C for 7 days was significantly decrease the virulence of some *B. basiana* isolates.

All isolates of *B. bassiana* were pathogenic against *N. lugens* nymphs. Mortality of the *N. lugens* nymphs caused by all isolates was high. However, virulence of *B. bassiana* isolates against *N. lugens* nymphs was significantly decrease if *B. bassiana* culture was incubated at 34 °C. Virulence of *B. bassiana* was decreased at 34 °C due to the decrease of conidial viability. Virulence of *B. bassiana* was affected by the conidial viability (El-Ghany, 2015). The higher of the capability of conidia germinate, the higher the probability of germ tube formation of the conidia penetrate insect cuticle (Butt et al., 1994; Fernandes et al., 2007). The local isolates from South Sumatra of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B in this study still had high conidial viability at 34 °C which in turn cause the high percentage of *N. lugens* mortality. These local isolates could adapt to high temperature of 34 °C and could be chosen as candidates for biocontrol agents of *N. lugens* in wetland or lowland rice ecosystems in Indonesia.

At fungal incubation temperature of 25 °C, mean of LT_{50} values ranged from 2.24 to 5.06 days and the shortest time was found on BTmPd isolate, whereas the longest time was found on 715 HHBanyuwangi isolate (Table 5). Mean of LT_{50} values caused by *B. bassiana* incubated at 34 °C ranged from 2.92 to 10.40 days and the shortest time was found on isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. They were isolates from South Sumatra. Whereas, the longest time was found on Bws Pantura isolate. TSID3B isolate culture incubated at 25ºC had the shortest lethal time values of 2.34 days (LT_{50}) and 7.16 days (LT_{95}). However, LT_{50} values were not significantly different among all isolates of *B. bassiana* and similar trend was also occurred on LT_{95}. Incubation temperature for *B. bassiana* culture affects LT_{50} or LT_{95} values. Mean of LT_{50} or LT_{95} values was significantly longer on *B. bassiana* incubated at 34ºC than that of *B. bassiana* incubated at 25ºC (Fig. 7). Therefore, incubation temperature at 34ºC for 7 days for *B. bassiana* culture could extend the lethal time to 50% and 95% mortality of *N. lugens* nymphs.

Incubation temperature at 34 °C for *B. bassiana* culture could prolong the LT_{50} and LT_{95} of *N. lugens* nymphs, but some isolates that still have short LT_{50} or LT_{95} value were consisted of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. LT_{50} or LT_{95} value of *N. lugens* caused by *B. bassiana* was affected by conidial viability of the fungus. Lower percentage of conidial germination cause longer time for the fungus to invade the whole body of insect hosts (Fernandes et al., 2007). In this research, the isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B at 34ºC causing the lethal time to 50% mortality of *N. lugens* nymphs were 3−4 days. The normal time required by conidia to kill insect host was 4−10 days (El-Ghany, 2015). The time required by *B. bassiana* to kill *N. lugens* nymphs in this study were shorter because insect hosts were more sensitive than that of other insect host species. Conidia requires certain time to germinate on insect cuticle surface and subsequently mycelia penetrates into body cavity (Fernandes et al., 2007). Then, the host insect will die whitin 4 days (Butt et al., 1994). Next, the fungus yields thousands of new spores on the dead body (El-Ghany, 2015).

CONCLUSION

It could be concluded from this study that at germination temperature of 34 °C, some isolates of *B. bassiana* originate from soils or insects, especially from South Sumatra, could produce high conidial density and viability as well as high virulent against *N. lugens* nymphs. The importance of this finding showed that some isolates were still virulent although their culture were incubated at 34 °C for 7 days. Therefore, the isolates can be used as promosing candidate for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as tidal lowland and lowland swamp ecosystems in Indonesia.

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Figures and Tables

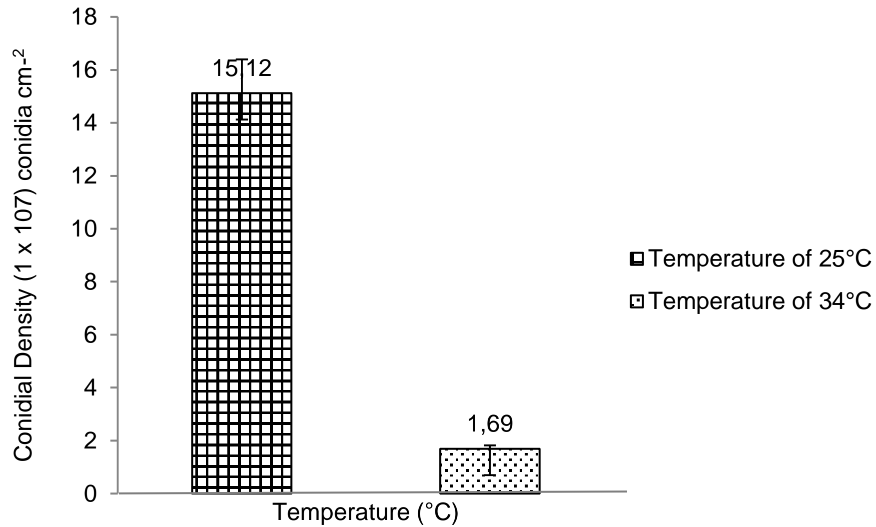


Fig. 1. Conidial density of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C (P = 0.00)

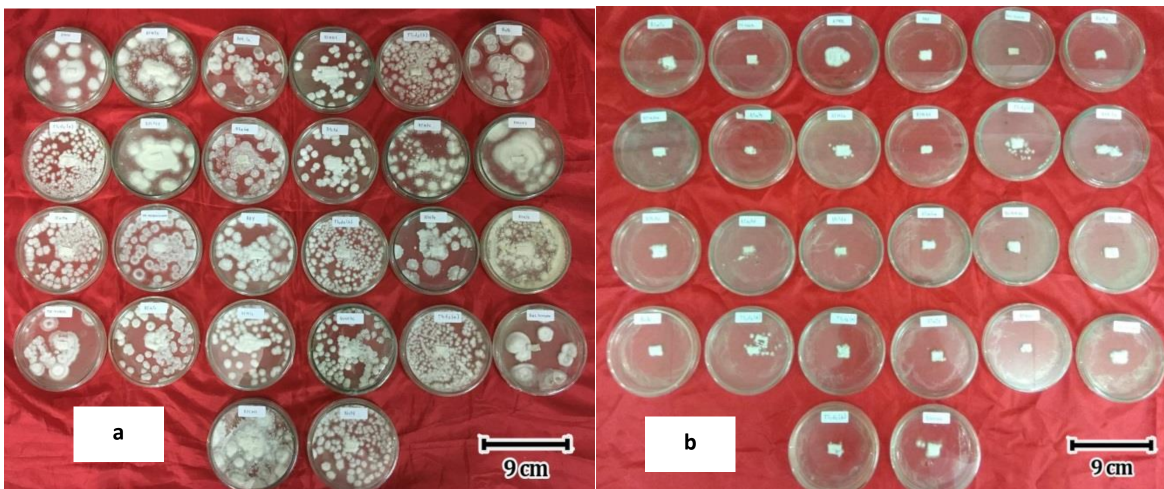


Fig. 2. Colony growth of *Beauveria bassiana* culture incubated for 7 days at 24 °C (a) dan 34 °C (b) (90 mm diameter of petri dish).

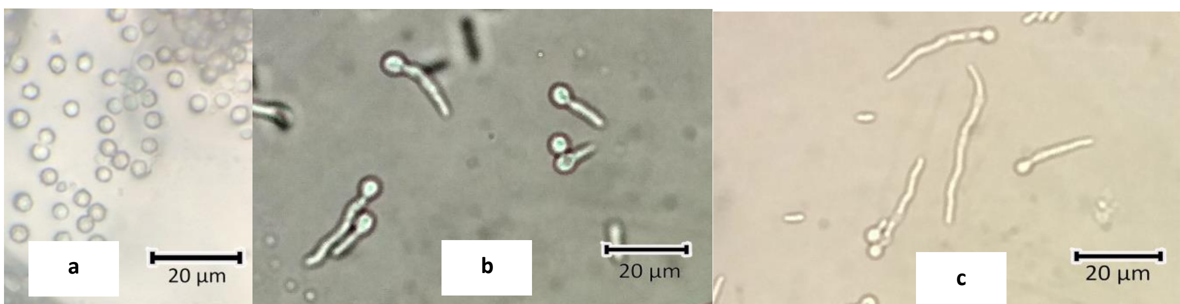
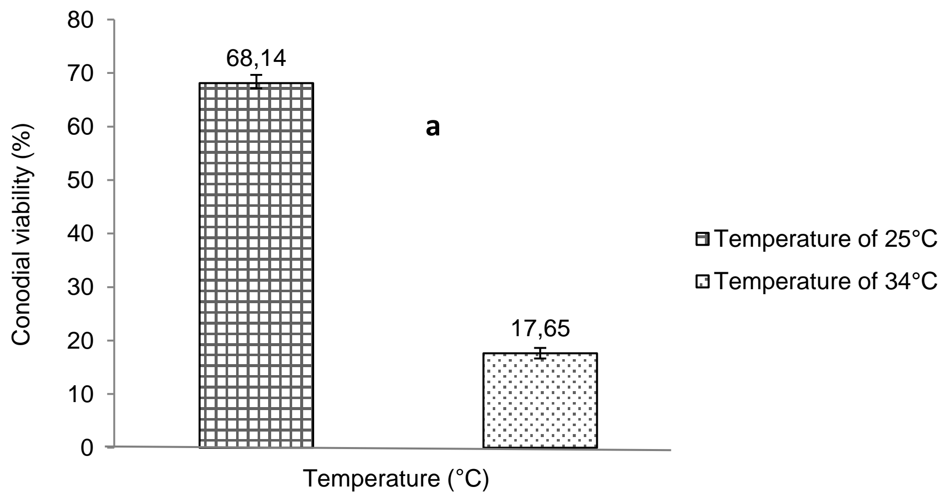


Fig. 3. Conidia of *Beauveria bassiana* (a), viable conidia at 24-hour (b) and 48-hours (c) suspension culture of *Beauveria bassiana* (400x magnification)



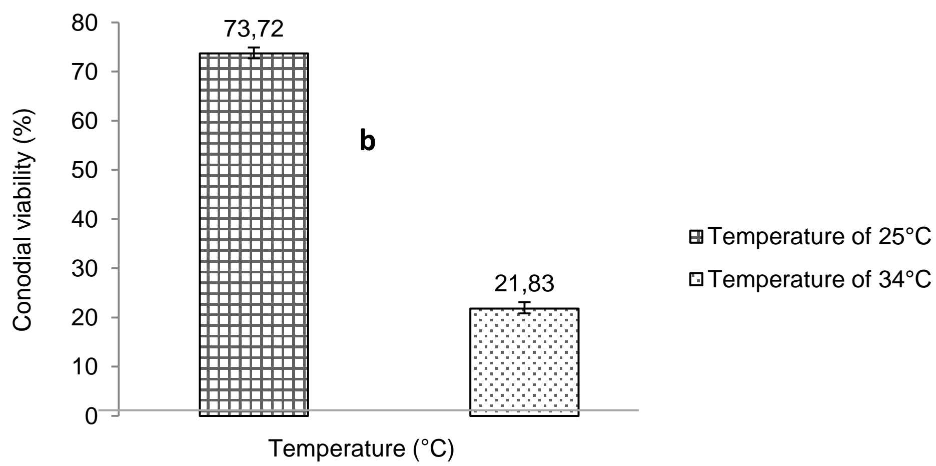


Fig. 4. Conidial viability of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C in 24-hour (P = 0.00) (a) and 48-hour suspension culture (P = 0.00) (b)

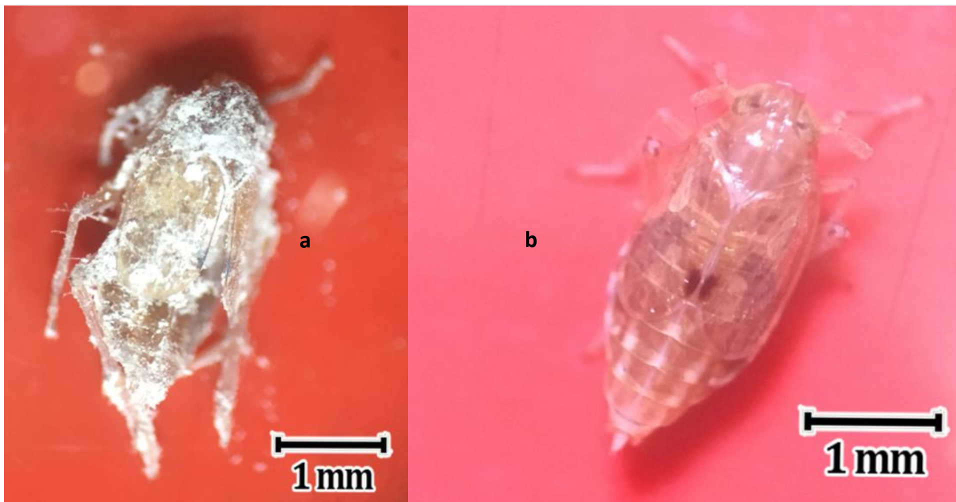


Fig. 5. *Nilaparvata lugens* infected by *Beauveria bassiana* (a) and the healthy one (b)

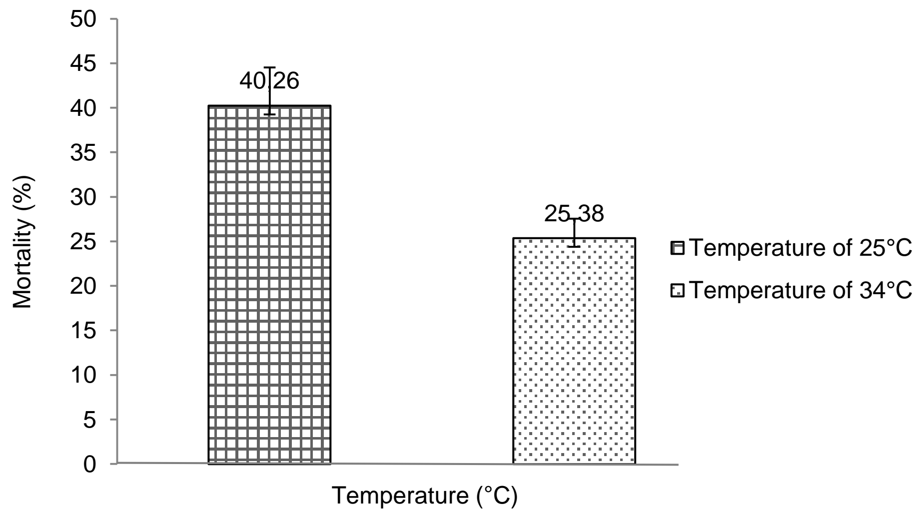
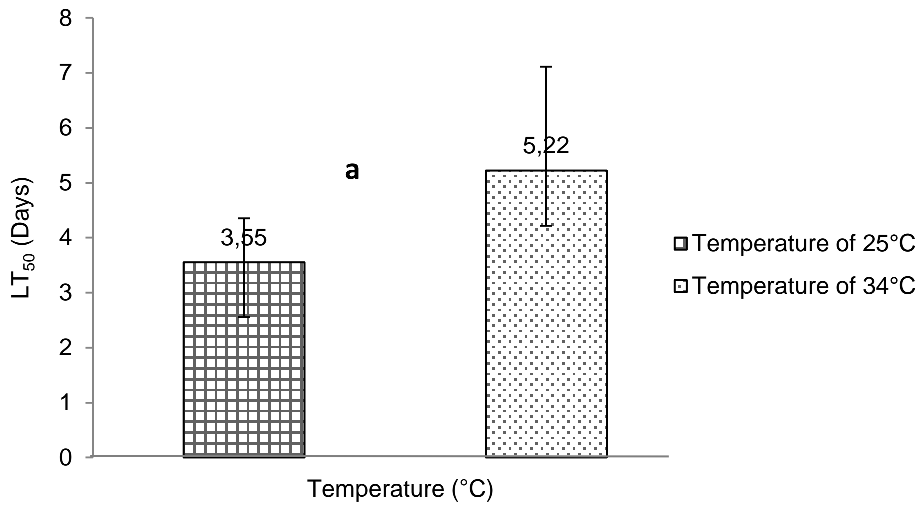


Fig. 6. Mortality of *Nilaparvata lugens* caused by *Beauveria bassiana* culture incubated at 25 and 34 °C (P = 0.003)



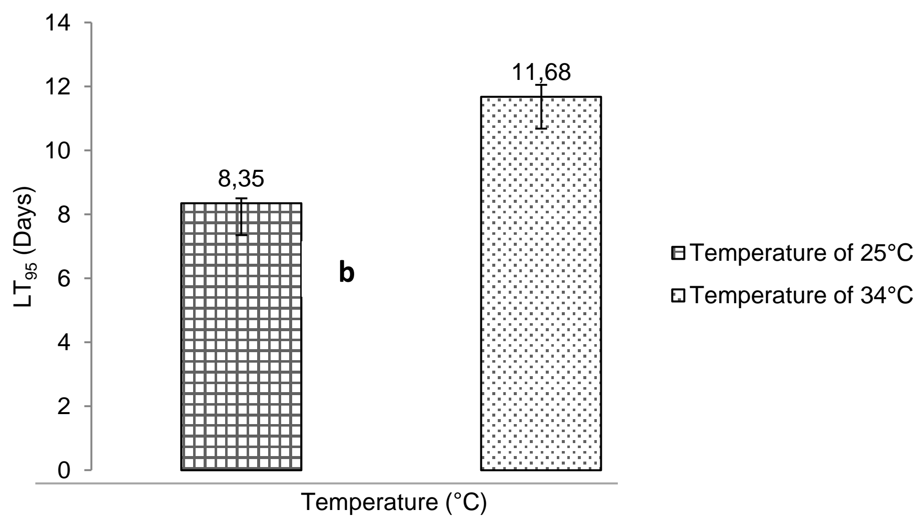


Fig. 7. LT_{50} (P = 0.00) (a) and LT_{95} (P = 0.00) (b) caused by *Beauveria bassiana* culture incubated at 25 and 34 °C against *Nilaparvata lugens*

Table 1. *Beauveria bassiana* isolates used in this research

Isolate codes	Species of fungi	Source (host insects or soil)	Origin (village or city), Province in Indonesia
BPcMs	*Beauveria bassiana*	*Pseudoplusia chalcites*	Muarasiban, South Sumatra
BTmKt	*Beauveria bassiana*	Fresh swamp soils	Kenten, South Sumatra
BTmPc	*Beauveria bassiana*	Fresh swamp soils	Indralaya, South Sumatra
Bws Pantura	*Beauveria bassiana*	*Leptocorisa acuta*	Pantura, West Java
BBY	*Beauveria bassiana*	*Hypothenemus hampei*	Jember, East Java
BTmPe	*Beauveria bassiana*	Fresh swamp soils	Pemulutan, South Sumatra
BTmMa	*Beauveria bassiana*	Fresh swamp soils	Mariana, South Sumatra
BTmSo	*Beauveria bassiana*	Fresh swamp soils	Soak, South Sumatra
BTmSr	*Beauveria bassiana*	Tidal lowland soils	Srikaton, South Sumatra
BuBj	*Beauveria bassiana*	*Alphitobius diaperinu*	Jarai, South Sumatra
725HaJ	*Beauveria bassiana*	*Helopeltis antonii*	Jember, East Java
715HhB	*Beauveria bassiana*	*Hypothenemus hampei*	Banyuwangi, East Java
BTmPd	*Beauveria bassiana*	Highland soils	Pagardin, South Sumatra
BTmTs	*Beauveria bassiana*	Highland soils	Mulia Sari, South Sumatra
BTmkbc	*Beauveria bassiana*	*Chrysodeixis chalcites*	Curup, Bengkulu
BPcPd2	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BPcPd	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BMkMs	*Beauveria bassiana*	Highland soils	Muarasiban, South Sumatra
BTmTr	*Beauveria bassiana*	Tidal lowland soils	Telang Rejo, South Sumatra
Natural BVR[#]	*Beauveria bassiana*	-	-
BTmGa	*Beauveria bassiana*	Fresh swamp soils	Gandus, South Sumatra
BLePd	*Beauveria bassiana*	*Lipaphis erysimi*	Pagardin, South Sumatra
TS1D3A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TSID3B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra

Remarks: [#]Control = commercial products, - unkown source

Table 2. Conidial density of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial density (10^7 conidia.cm^{-2})	
	25 °C	**34 °C**
BPcMs	27.34 m	2.71 bcd
BTmKt	9.81 bcde	1.19 abcd
BTmPc	13.01 efgh	1.28 abcd
Bws Pantura	16.87 hijk	1.61 abcd
BBY	19.25 jkl	1.27 abcd
BTmPe	12.26 defg	1.04 ab
BTmMa	10.93 cdef	1.15 abcd
BTmSo	28.21 m	2.30 abcd
BTmSr	6.76 a	1.50 abcd
BuBj	11.42 cdef	1.83 abcd
725HaJ	16.26 ghij	1.09 abc
715HhB	7.73 ab	1.11 abc
BTmPd	11.35 cdef	1.43 abcd
BTmTs	12.49 efgh	1.45 abcd
BTmkbc	14.17fghi	1.33 abcd
BPcPd2	9.40 bcd	0.97 a
BPcPd	9.87 bcde	1.73 abcd
BMkMs	14.45 fghi	1.20 abcd
BTmTr	11.79 efg	1.24 abcd
Natural BVR[#]	11.72 efg	1.55 abcd

BTmGa	13.07 efgh	1.54 abcd
BLePd	8.44 abc	1.45 abcd
TS1D3A	21.14 jklm	3.03 d
TSID3B	22.82 klm	2.80 cd
TS1D2A	28.46 m	3.00 cd
TS1D2B	24.02 lm	3.05 d
ANOVA F-value	49,05*	3.98*
P value (0.05)	$1,7 \times 10^{-37}$	1.61×10^{-6}
Tukey's HSD test	0,1383	0.4184

Remarks: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to HSD test

Table 3. Conidial viability of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	**Conidial viability (%) of 24-hour-suspension culture**		**Conidial viability (%) of 48-hour-suspension culture**	
	25 °C	**34 °C**	**25 °C**	**34 °C**
BPcMs	70.17 ab	15.54 bcdef	77.46	26.05 hijk
BTmKt	47.77 a	16.72 bcdefg	58.38	24.75 fghijk
BTmPc	66.46 ab	26.30 hi	76.35	28.54 jkl
Bws Pantura	66.51 ab	9.09 ab	75.48	10.57 ab
BBY	73.02 ab	23.56 ghi	75.05	26.05 hijk
BTmPe	63.44 ab	22.28 fghi	70.85	25.49 ghijk
BTmMa	67.94 ab	14.26 bcde	72.68	14.26 bcd
BTmSo	74.83 ab	19.71 cdefgh	77.55	22.47 fghi
BTmSr	73.62 ab	20.99 defgh	75.47	20.99 efgh
BuBj	74.30 ab	21.64 efghi	78.69	22.92 fghij
725HaJ	66.49 ab	14.03 bcd	72.10	16.63 cde
715HhB	68.77 ab	11.20 abc	74.43	13.32 bc
BTmPd	75.19 ab	23.77 ghi	76.45	28.52 jkl
BTmTs	76.30 ab	12.40 abc	79.85	12.40 bc
BTmkbc	66.24 ab	22.51 fghi	70.43	22.51 fghi
BPcPd2	71.44 ab	7.98 a	70.42	7.98 a
BPcPd	63.60 ab	15.10 bcdef	69.81	15.10 bcd
BMkMs	65.06 ab	14.42 bcde	72.14	21.53 efgh
BTmTr	70.00 ab	15.44 bcdef	73.70	24.02 ghijk
Natural BVR[#]	60.74 ab	28.53 i	69.73	32.58 l
BTmGa	63.42 ab	19.89 cdefgh	67.15	19.89 defg
BLePd	48.80 a	17.17 cdefg	59.00	19.31 def
TS1D3A	79.33 b	17.75 cdefg	81.42	25.61 ghijk
TSID3B	69.49 ab	15.87 bcdef	80.23	28.72 jkl
TS1D2A	78.41 b	17.69± cdefg	81.38	28.12 ijkl
TS1D2B	70.29 ab	15.14 bcdef	80.58	29.22 kl
ANOVA F-value	2,02*	3,4*	2,03ns	5.89*
P value (0.05)	10×10^{-8}	4.6×10^{-6}	$9,7 \times 10^{-10}$	9×10^{-10}
Tukey's HSD test	18,057	7.45	-	7.56

Remarks: ns = not significantly different; * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to HSD test

Table 4. Virulence of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C against *Nilaparvata lugens* nymphs

Isolate codes	**Mortality of** *Nilaparvata lugens* nymphs **(%)**	
	25 °C	**34 °C**
BPcMs	65.00	21.67
BTmKt	26.67	13.33
BTmPc	43.33	28.33
Bws Pantura	30.00	5.00

BBY	38.33	16.67
BTmPe	28.33	21.67
BTmMa	28.33	25.00
BTmSo	56.67	40.00
BTmSr	35.00	35.00
BuBj	45.00	26.67
725HaJ	23.33	20.00
715HhB	15.00	6.67
BTmPd	26.67	20.00
BTmTs	26.67	13.33
BTmkbc	28.33	28.33
BPcPd2	20.00	10.00
BPcPd	25.00	25.00
BMkMs	26.67	26.67
BTmTr	31.67	20.00
Natural BVR[#]	43.33	36.67
BTmGa	30.00	30.00
BLePd	26.67	23.33
TS1D3A	90.00	38.33
TSID3B	58.33	43.33
TS1D2A	81.67	41.67
TS1D2B	96.67	43.33
ANOVA F-value	0.29ns	0.16ns
P value (0.05)	0.99	1.00

Remarks: ns = not significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to HSD test

Table 5. LT_{50} and LT_{95} of *Nilaparvata lugens* nymphs caused by *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	LT_{50} (days) (95% fiducial limits)		LT_{95} (days) (95% fiducial limits)	
	25 °C	34 °C	25 °C	34 °C
BPcMs	2.62 (1.68 – 3.72)	4.95 (3.71 – 6.14)	7.43 (5.66 – 9.15)	11.40 (9.43 – 12.78)
BTmKt	4.18 (3.37 – 4.82)	7.48 (5.77 – 8.76)	9.00 (8.35 – 10.25)	13.93 (11.63 – 16.56)
BTmPc	3.86 (2.58 – 5.28)	4.87 (4.26 – 5.20)	8.58 (7.71 – 10.03)	11.33 (9.97 – 13.00)
Bws Pantura	4.31 (2.86 – 6.85)	10.40 (7.22 – 14.22)	9.13 (8.26 – 10.83)	16.85 (12.94 – 22.01)
BBY	2.93 (2.60 – 3.33)	5.96 (3.62 – 8.12)	7.75 (6.84 – 8.76)	12.41 (9.34 – 15.91)
BTmPe	3.88 (3.65 – 4.23)	5.92 (3.11 – 10.56)	8.69 (8.21 – 9.08)	12.37 (8.96 – 18.36)
BTmMa	2.76 (2.01 – 4.16)	2.99 (1.36 – 4.42)	7.57 (6.09 – 9.19)	9.45 (7.08 – 12.22)
BTmSo	3.31 (2.68 – 4.42)	3.09 (2.56 – 3.68)	8.13 (7.71 – 8.40)	9.55 (8.27 -11.48)
BTmSr	2.57 (1.94 – 2.95)	2.92 (2.40 – 3.60)	7.39 (6.94 – 8.26)	9.38 (8.11 – 10.56)
BuBj	2.68 (1.76 – 4.16)	6.43 (1.45 – 10.35)	7.49 (6.09 – 9.19)	12.89 (7.17 – 18.14)
725HaJ	3.19 (2.73 – 3.76)	5.65 (4.35 – 7.04)	8.00 (7.05 – 8.79)	12.00 (11.41 – 12.45)
715HhB	5.06 (2.98 – 6.76)	9.00 (6.92 – 10.47)	9.88 (6.96 – 12.19)	15.46 (12.77 – 17.42)
BTmPd	2.24 (1.59 – 3.24)	5.02 (2.73 – 8.85)	7.06 (5.88 – 8.67)	11.47 (8.44 – 16.65)
BTmTs	3.93 (3.07 – 4.98)	6.55 (5.44 – 8.12)	8.75 (7.05 – 10.40)	13.01 (11.15 – 15.91)
BTmkbc	4.61 (3.24 – 5.32)	3.31 (1.21 – 6.13)	9.13 (7.77 – 10.31)	9.76 (6.92 – 13.93)
BPcPd2	4.21 (2.73 – 6.82)	8.15 (2.75 – 14.22)	9.02 (7.05 – 11.85)	14.61 (8.46 – 22.01)
BPcPd	4.39 (3.09 – 5.65)	4.37 (3.09 – 6.51)	9.20 (8.40 – 10.68)	10.91 (8.80 – 12.64)
BMkMs	4.19 (2.66 – 7.09)	3.95 (2.23 – 6.65)	9.01 (6.64 – 12.12)	10.74 (7.95 – 12.51)
BTmTr	3.82 (2.91 – 4.65)	4.66 (3.97 – 5.09)	8.64 (6.89 – 10.08)	11.12 (9.82 – 12.72)
Natural BVR[#]	4.12 (3.37 – 5.44)	4.06 (3.65 – 4.65)	8.94 (7.35 – 10.47)	10.51 (9.71 – 11.45)
BTmGa	3.68 (2.93 – 4.36)	3.96 (2.94 – 4.66)	8.50 (8.35 – 8.79)	10.41 (8.66 – 12.08)
BLePd	3.90 (3.33 – 4.59)	3.79 (3.50 – 3.97)	8.72 (8.36 – 9.22)	10.25 (9.62 – 11.29)
TS1D3A	2.99 (2.46 – 3.55)	4.17 (4.07 – 4.32)	7.80 (6.94 – 8.98)	10.63 (9.79 – 11.92)
TSID3B	2.34 (1.63 – 3.72)	3.92 (3.23 – 4.91)	7.16 (5.61 – 9.15)	10.38 (9.48 – 11.02)
TS1D2A	2.41 (1.19 – 3.10)	5.80 (4.17 – 7.26)	7.23 (6.62 – 7.97)	12.26 (9.88 – 13.78)
TS1D2B	4.19 (3.05 – 5.28)	4.35 (3.48 – 4.82)	9.01 (8.23 – 10.31)	10.67 (10.13 -11.28)
ANOVA F-value	0.84ns	2.08ns	0.84ns	1.21ns

P value (0.05)	0.68	0.01	0.68	0.28

Remarks: ns = not significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to HSD test

2. Bukti konfirmasi review pertama dan hasil revisi pertama

Editor Decision

AGRIVITA <agrivita@ub.ac.id> Sen, 13 Mei 2019, 14.04
kepada saya, Eka, Yulia

Dear Siti Herlinda,

We have reached a decision regarding your submission to AGRIVITA, Journal of Agricultural Science, "Conidial Density and Viability of Beauveria bassiana Isolates from Indonesia and their Virulence against Nilaparvata lugens at Different Temperatures".

Based on the review result, we decided to return the manuscript for revision. We agree with the reviewer comments especially the research focus and data analysis.

We look forward the revision of manuscript.

Sincerely,

The revised manuscript of ID 2105

Prof. Dr. Siti Herlinda <sitiherlinda@unsri.ac.id> Kam, 16 Mei 2019, 07.50
kepada arizali, Agrivita

Dear Editor,
AGRIVITA
Journal of Agricultural Science

We have revised our manuscript and the revisions were marked with red letters. We also submitted the revision at OJS of Agrivita. Please find the revised manuscript attached. Thanks

Best Regards
Prof. Dr. Siti Herlinda

Satu lampiran • Dipindai dengan Gmail

Conidial Density and Viability of *Beauveria bassiana* Isolates from Java and Sumatra and their Virulence against *Nilaparvata lugens* at Different Temperatures

ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* can cause direct damage and transmit rice diseases, such as ragged and grassy stunt virus. *Beauveria bassiana* was used to control BPH, however the success of the fungal efficacy on rice fields was affected by external factors, such as temperature. This research aimed to evaluate the conidial viability and density of *B. bassiana* isolates from Java and Sumatra exposed to 25 and 34 °C and their virulence against BPH nymphs. Twenty six isolates from Java and Sumatra of *B. bassiana* cultures incubated at 25 and 34 °C for 7 days were observed their conidial density, viability, and virulence against BPH nymphs. Incubation temperature of 34 °C was able to decrease conidial density and viability, and virulence of the isolates. However, some isolates of *B. bassiana* originated from soils or insects in Sumatra, especially from South Sumatra still produced high conidial density and viability as well as high virulent against BPH nymphs, such as TS1D3A, TSID3B, TS1D2A and TS1D2B isolates. The TS1D2B isolate incubated at 34 °C still caused the highest percentage of BPH mortality (43.33%) among other isolates. Therefore, the isolates can be used as promosing candidate for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as wetland or lowland rice ecosystems in Indonesia.

KEYWORDS

Biocontrol, entomopathogenic fungus, brown planthopper, mortality

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae) is the most serious insect pest which sucks phloem sap on stems of rice (*Oryza sativa* L.) (Daravath & Chander, 2017; Herlinda et al., 2018). The direct damage caused by this BPH results in hampered growth of rice which in turn produce of 'hopperburn' (Dharshini & Siddegowda, 2015). In addition, BPH can also as an insect vector that transmits rice diseases, such as the ragged stunt and grassy stunt virus (Zheng et al., 2014; Dietzgen et al., 2016). The attack by this BPH not only occured in Indonesia, but also had attacked rice in several Asian countries, such as China, Vietnam, Thailand, India, Pakistan, Malaysia and Philippine (Catindig et al., 2009; Hu et al., 2014). BPH had also attacked rice in Texas (Leavengood et al., 2017).

The effort to control population of *N. lugens* had been done through synthetic chemical control (Liu et al., 2013; Zhang et al., 2014; Baehaki & Suparno, 2018) and biological control by using entomopathogenic fungi, such as *Beauveria bassiana* (Li et al., 2012, 2014; Lee et al., 2015) and *Metarhizium anisopliae* (Chinniah et al., 2016) that had proven to be an effective agents to control the BPH. *B. bassiana* could kill the BPH more than 80% (Li et al., 2014; Lee et al., 2015) and to kill eggs of the BPH as well as to disturb

adult stage proliferation of the BPH (Li et al*.,* 2012). *B. bassiana* is also not harmful toward natural enemies of the BPH (Firouzbakht et al., 2015; Gholamzadeh-Chitgar et al., 2017).

Although *B. bassiana* had proven to be effective in controlling the BPH, the success of its efficacy on rice fields was affected by many external factors, such as temperature (El-Ghany, 2015). Extremely high temperature can results in death of the fungus (Ottati-de-lima et al., 2014). The optimum temperature for growth of the entomopathogenic fungi usually is in the range of 25 to 30ºC (Bugeme et al., 2008). Most of entomopathogenic fungi tolerate to temperatures in the range of 0 to 40°C (El-Ghany, 2015), but certain strains of entomopathogenic fungi can only survive at temperatures below 35 °C (Constanski et al., 2011). Production of colony and conidial density of *B. bassiana* is significantly decrease if temperature during fungal incubation is increased from 30 to 35 °C (Ottati-de-lima et al., 2014) and all isolates are death at temperature of 36 °C (Pham et al., 2009; Ottati-de-lima et al., 2014). Fungal germination is also decrease at temperature above 30 °C (Pham et al., 2009) with the highest level up to 33 °C (Salim et al., 2015), whereas temperature of 25°C is an ideal temperature for the fungal germination (Lohse et al., 2014). Virulence of the entomopathogenic fungi can be affected by temperature (Tefera & Pringle, 2003; Bugeme et al., 2008; Constanski et al., 2011; Ottati-de-lima et al., 2014; El-Ghany, 2015; Satpathi et al., 2016). Each strain/isolate or species of the entomopathogenic fungi also has different optimum temperature and the tolerance level to temperature. Entomopathogenic fungal strains that could survive at extremely high temperature of above 33 °C are superior strains (Salim et al., 2015). These superior strains can be used as candidates to control the BPH in tropical ecosystems, such as agroecosystems in Indonesia. Therefore, the objectives of this research were to evaluate the conidial viability and density of *B. bassiana* isolates form Java and Sumatra exposed to 25 and 34 °C temperatures and their virulence against *N. lugens* nymphs.

MATERIALS AND METHODS

Study Site

This research was conducted at Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, from September 2017 to April 2018. Isolates used in this research were isolates collected from soil of lowland swamps, tidal lowlands, peatlands, and highlands in South Sumatra, whereas isolates from soil and infected insects obtaining from other provinces were used as comparison as well as one commercial isolate was used as control (Table 1). The species of the fungus was identified by Dr. Suwandi, a microbiologist from Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya. Average room temperature during experiment was 30.50 °C and average relative humidity was 84.30% within the laboratory during the experiment.

Preparation of Test Insects

Adults and nymphs of *N. lugens* were collected from fields of rice at Indralaya, South Sumatra since September 2017 up to March 2018 and brought to Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya for identification. Then, the nymph and adults were reared and maintained on 5 clumps of 10-day-old rice grown within bucket (bottom diameter of

20 cm, upper diameter of 25 cm, and height of 20 cm) within a greenhouse at temperature range of 30 to 35 °C. The rice was put in wire mesh enclosure (height of 100 cm, length of 50 cm, and width of 50 cm). Within one of wire mesh enclosure, 10 pairs of the adults were released in order to infect the rice for 10 days and subsequently the infected rice crops were substituted with the healthy one and this propagation was done on 30 cages. Fresh and healthy rice were given to newly emerged nymphs of *N. lugens* and this was maintained up to at least of five generations. The sixth generation and henceforth generations were used and selected for the fourth instar for bioassay in this experiment.

Isolates Preparation of *Beauveria bassiana*

All isolates used in this study were previously fitted by using living insects of *Tenebrio molitor.* Fresh cultures of *B. bassiana* were started by inoculating Sabouraud Dextrose Agar (SDA) (Oxoid) with the third larvae of *T. molitor*. Prior to inoculation, *B. bassiana* and *T. molitor* were previously sterilized by using modified method from Nuraini et al. (2017). Subsequently, the fungal culture was incubated for 7 x 24 hours in order to produce sufficients numbers of fungal colony. The *B. bassiana* culture that had previously fitted was then used for subsequent observation. The fresh *B. bassiana* culture was cut with dimension of 10 mm x 10 mm for recultured into SDA medium which would be used for subsequent observation consisting of conidial density and viability as well as virulence test.

Observation of Conidial Density and Viability

Twenty six isolates of the *B. bassiana* culture were grown on SDA medium and then each isolate was incubated at constant temperature of 25 and 34 °C within incubator for 7 x 24 hours using three replications. The ideal temperature of 25 °C and extremely high temperature of 34 °C in this research were chosen for culturing of *B. bassiana* for 7 days. Both temperature were chosen because temperature of 25 °C is ideal temperature for culture *B. bassiana* (Bugeme *et al.*, 2008), whereas temperature higher than 33 °C is extremely high temperature (Salim et al., 2015). Conidia of all isolates at the 8th day were counted in term of their density of the 7 day *B. bassiana* culture. Calculation of conidial density was started with fungal suspension production by harvesting 10 mm x 10 mm (1 cm^2) 7-day *B. bassiana* culture which followed by 10 mL addition of sterile distilled water. The suspension was vortexed using turbo mixer for 20 seconds in order to produce homogenous conidial suspension. This suspension culture was diluted through addition of 9 mL sterile distilled water into 1 mL *B. bassiana* suspension culture, homogenized. Subsequently, the last suspension culture was counted in term of its conidial density under a compound microscope at 400x magnification that had been equipped with haemocytometer. This treatments were arranged in completely randomized design and replicated three times.

Calculation of conidial viability was based on percentage of conidial germination. The conidial germination was observed from the 7-day *B. bassiana* culture which incubated at 25 or 34 °C and grown on one layer of SDA medium according to the method of Bugeme et al. (2008). The 7-day *B. bassiana* suspension culture was scratched with magnitude of 0.1 mL on SDA plates. A sterile microscope cover slip was placed on each plate, then each suspension culture was incubated for 24 and 48 hours at temperature of 25 °C. Furthermore, the germinated conidia was observed under microscope and calculation was done in term of numbers of germinated conidia. The germination percentage was determined from 100-spores for

each plate using the compound microscope. These treatments were arranged in completely randomized design and replicated three times.

Bioassay Procedure

Bioassays by modification of Herlinda (2010) and Trizelia and Nurdin (2010) method were conducted to determine the virulence of *B. bassiana* isolates againts *N. lugens* nymphs. The 26 isolates of *B. bassiana* were incubated at 25 and 34 °C for 7 days and their conidia were harvested. Each isolates of *B. bassiana* was topically sprayed with 10 ml of a concentration of 1 x 10^3 conidia cm^{-2} on 25 fourth-nymphs of *N. lugens* placed on a filter paper in petri dishes. Then, the nymphs of *N. lugens* were placed into 20 healthy two-weeks rice stems. This treatments were arranged in completely randomized design and replicated three times. Numbers of dead *N. lugens* nymphs was recorded every 12 hours for 11 days period which used to determine the percentage of mortality and lethal time to 50% (LT_{50}) and 95% (LT_{95}) mortality of *N. lugens* nymphs. The behaviour change of *N. lugens* infested by *B. bassiana* was observed and recorded daily until the insects were death and mycelia cover all of their bodies. The dead nymphs were transferred into petri dishes lined with moist-sterile filter paper to allow the growth of the *B. bassiana* on the surface of the cadaver.

Data Analysis

Data of conidial density and viability, and percentage of mortality among treatments were analyzed by using analysis of variance (ANOVA). If there were differences among the data of each isolate, then Honestly Significant Different (HSD) test at 5% was conducted by using program software of SAS University Edition 2.7 9.4 M5. The data between temperature were were compared by using t test. LT_{50} and LT_{95} values were calculated by using probit analysis.

RESULTS AND DISCUSSION

Conidial Density and Viability of *Beauveria bassiana*

B. bassiana culture incubated for 7 days at temperature of 25 °C showed that the highest conidial density was found on BTmSo isolate and was not significantly different than that of BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B isolates (Table 2). At incubation temperature of 34 °C, the highest value of conidial density was found on TS1D2B isolate and was significantly different than that of BPcPd2 isolate which had the lowest conidial density value. Conidial density of all isolates at 25 °C were siginificantly higher than that of the isolates at 34 °C (P = 0.00) (Fig. 1). Conidial density significantly decreased with the increase of the fungal incubation temperature and followed by more hampered of fungal colony growth. Fungal culture incubated for 7 days at 25 °C had normal growth with colony diameter in the range of 50 to 90 mm, whereas colony of *B. bassiana* incubated at 34 °C only had diameter in the range of 15 to 30 mm (Fig. 2). Incubation temperature of 34 °C for *B. bassiana* culture was able to decrease conidial density and colony growth of the fungus; however some isolates (TS1D3A, TSID3B, TS1D2A and TS1D2B isolates) were still achieved high conidial density and colony growth.

Conidial density values of *B. bassiana* isolate cultures incubated at 25 °C were all high, but higher values were found on isolates of BtmSo, BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B. They were isolates from South Sumatra. Conidial density of all isolates were siginificantly decrease if the isolates were incubated at 34 °C. However, only TS1D2B isolate still had high conidial density at incubation temperature of 34 °C. The conidial density was decrease at 34 °C due to lower production of conidia cm^{-2} in agar medium which indicated by hampered colony growth of *B. bassiana* (Fig. 2). The diameter of colony growth reach 90 mm at 25 °C, whereas diameter of colony growth at 34 °C was only 15-30 mm. Ottati-de-lima et al. (2014) had stated that *M. anisopliae* could yield optimal colonies in liquid medium from 25 to 30 °C and temperature of 35 °C was detrimental to colony growth of the fungus. High spore or conidial production of *B. bassiana* was occurred at 25-27 °C (Pham et al., 2009). Incubation temperature of 34 °C in this research could decrease the conidial density and colony growth of *B. bassiana*.

Viable or germinate conidia was characterized by the following aspects: the change of size and form of conidia compared to size and form of normal conidia (Fig. 3a), conidial wall was broken and produce germ tube and followed by elongation of the germ tube (Fig. 3b and 3c). Percentage of conidial germination was used to determine conidial viability. At incubation temperature of 25 °C, the highest value of conidial viability of 24-hour-suspension culture was found on TS1D3A isolate and was not significantly different than that of other isolates, except BTmKt and BLePd isolates (Table 3). However, the highest value of conidial viability at temperature of 34 °C was found on Natural BVR# isolate in control of commercial product and was not significantly different than that of other five isolates consisting of BTmPc, BBY, BtmPe, BTmPd and BTmkbc. Conidial viability of 48-hour-suspension culture at 25 °C for all isolates were high and was not significantly different among isolates. Nevertheless, isolates that had the highest conidial viability at 34 °C was Natural BVR# isolate and was not significantly different than that of BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B isolates. Temperature increase during incubation of *B. bassiana* had significant effect on conidial viability (Fig. 4). Conidial viability was significantly decrease if the *B. bassiana* culture was incubated at 34 °C, either for 24-hour-suspension culture ($P = 0.00$) or 48-hour-suspension culture ($P = 0.00$). Therefore, incubation temperature at 34 °C for 7 days for *B. bassiana* culture was capable to decrease the fungal conidial germination, although some local isolates of BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B could produce high percentage of germination.

The shape of conidial germination of *B. bassiana* in this research was similar to description of conidial germination given by the following researcher. Conidial germination showed the following signs: broken conidial wall resulting in germ tube formation which had long stretch and increase in size or diameter of conidia (Guilherme et al., 2015; Safitri et al., 2018). Percentage of conidial germination was used to measure conidial viability of the fungus. Conidial viability of *B. bassiana* at 25 °C was high on TS1D3A, BTmKt and BLePd isolates. However, the conidial viability that was still high was found on TS1D3A, commercial BVR#, BTmPc, BBY, BTmPd, TSID3B and TS1D2B isolates at 34ºC. The conidial viability of *B. bassiana* in 24-hour suspension or 48-hour suspension culture was decrease significantly at incubation temperature of 34 °C. Ideal temperature for the conidial germination of *B. bassiana* was in the range of 25 to 27 °C, but *B. bassiana* conidia could still germinate at 32 °C (Constanski et al., 2011). Salim et al. (2015) had stated that the conidial germination of *B. bassiana* was still occurred up to 33 °C. Local isolates of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B originated from soils and insects of South Sumatra and East

Java, Indonesia were still had high conidial viability at 34 °C. The conidial viability of these local isolates were equivalent to those of the commercial BVR# isolate. The conidial isolates that were capable to germinate at 34 °C were rarely occurred, but only high temperature resistant isolates that had capability to germinate. According to Salim et al. (2015) only superior strains of the fungi were able to germinate at above 33ºC. The conidia of the local isolates in this experiment that were capable to germinate at 34ºC had potential to be developed as active ingredients of bioinsecticides to control *N. lugens* in high temperature rice ecosystem, such as wetland or lowland rice ecosystems of South Sumatra, Indonesia. According to Lakitan et al. (2018a,b) temperature occured in wetland or lowland rice ecosystems of South Sumatra was above 30 °C.

Virulence of *Beauveria bassiana*

The symptom of *N. lugens* nymphs infected by *B. bassiana* was started to appear at second day after being exposed to the fungal conidia with doses of $1x10^3$ conidia cm^{-2}. The nymphs had slow movement and finally stop moving, whereas the healthy nymphs still actively moving at rice stem. On the third day, some of the sick nymphs were death, whereas the other infected nymphs which were still alive became unable to move anymore with their legs and stylets attached on rice stem. On the fourth and fifth days, the dead nymphs hung with their stylets still attached on rice stem, whereas all their legs were not grip on rice stem anymore. On the sixth day, the dead nymphs became hardened and stiff. On the seventh day, their bodies wrinkle, decay with no smell and their integuments were coated with white color mycelia which gradually become brownish white to dark brown colors (Fig. 5).

Virulence of *B. bassiana* isolates against *N. lugens* nymphs was represented on percentage of mortality and the lethal time to 50% (LT_{50}) and 95% (LT_{95}) mortality of *N. lugens* nymphs caused by *B. bassiana.* All isolates of *B. bassiana* tested in this experiment were pathogenic against the *N. lugens* nymphs. At temperature of 25 °C, mean mortality of *N. lugens* nymphs with magnitude of 96.67% was found on TS1D2B isolate, whereas the lowest mean mortality with magnitude of 20% was found on BPcPd2 isolate (Table 4). The highest value of mean mortality of *N. lugens* nymphs at 34 °C was found on TS1D2B isolate (43.33%), wehereas the lowest value of mean mortality of *N. lugens* nymphs was found on Bws Pantura isolate (5%). However, mortality of *N. lugens* nymphs caused by *B. bassiana* was not significantly different among all isolates either at temperarture of 25 or 34 °C. The percentage of *N. lugens* mortality was significantly decrease if fungal incubation temperature was increased from 25 to 34 °C (P = 0.03) (Fig. 6). Therefore, incubation temperature at 34 °C for 7 days was significantly decrease the virulence of some *B. basiana* isolates.

All isolates of *B. bassiana* were pathogenic against *N. lugens* nymphs. Mortality of the *N. lugens* nymphs caused by all isolates was high. However, virulence of *B. bassiana* isolates against *N. lugens* nymphs was significantly decrease if *B. bassiana* culture was incubated at 34 °C. Virulence of *B. bassiana* was decreased at 34 °C due to the decrease of conidial viability. Virulence of *B. bassiana* was affected by the conidial viability (El-Ghany, 2015). The higher of the capability of conidia germinate, the higher the probability of germ tube formation of the conidia penetrate insect cuticle (Butt et al., 1994; Fernandes et al., 2007). The local isolates from South Sumatra of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B in this study still had high conidial viability at 34 °C which in turn cause the high percentage of *N. lugens*

mortality. These local isolates could adapt to high temperature of 34 °C and could be chosen as candidates for biocontrol agents of *N. lugens* in wetland or lowland rice ecosystems in Indonesia.

At fungal incubation temperature of 25 °C, mean of LT_{50} values ranged from 2.24 to 5.06 days and the shortest time was found on BTmPd isolate, whereas the longest time was found on 715 HHBanyuwangi isolate (Table 5). Mean of LT_{50} values caused by *B. bassiana* incubated at 34 °C ranged from 2.92 to 10.40 days and the shortest time was found on isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. They were isolates from South Sumatra. Whereas, the longest time was found on Bws Pantura isolate. TSID3B isolate culture incubated at 25ºC had the shortest lethal time values of 2.34 days (LT_{50}) and 7.16 days (LT_{95}). However, LT_{50} values were not significantly different among all isolates of *B. bassiana* and similar trend was also occurred on LT_{95}. Incubation temperature for *B. bassiana* culture affects LT_{50} or LT_{95} values. Mean of LT_{50} or LT_{95} values was significantly longer on *B. bassiana* incubated at 34ºC than that of *B. bassiana* incubated at 25ºC (Fig. 7). Therefore, incubation temperature at 34ºC for 7 days for *B. bassiana* culture could extend the lethal time to 50% and 95% mortality of *N. lugens* nymphs.

Incubation temperature at 34 °C for *B. bassiana* culture could prolong the LT_{50} and LT_{95} of *N. lugens* nymphs, but some isolates that still have short LT_{50} or LT_{95} value were consisted of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. LT_{50} or LT_{95} value of *N. lugens* caused by *B. bassiana* was affected by conidial viability of the fungus. Lower percentage of conidial germination cause longer time for the fungus to invade the whole body of insect hosts (Fernandes et al., 2007). In this research, the isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B at 34ºC causing the lethal time to 50% mortality of *N. lugens* nymphs were 3−4 days. The normal time required by conidia to kill insect host was 4−10 days (El-Ghany, 2015). The time required by *B. bassiana* to kill *N. lugens* nymphs in this study were shorter because insect hosts were more sensitive than that of other insect host species. Conidia requires certain time to germinate on insect cuticle surface and subsequently mycelia penetrates into body cavity (Fernandes et al., 2007). Then, the host insect will die whitin 4 days (Butt et al., 1994). Next, the fungus yields thousands of new spores on the dead body (El-Ghany, 2015).

CONCLUSION

It could be concluded from this study that at germination temperature of 34 °C, some isolates of *B. bassiana* originate from soils or insects, especially from South Sumatra, could produce high conidial density and viability as well as high virulent against *N. lugens* nymphs. The importance of this finding showed that some isolates were still virulent although their culture were incubated at 34 °C for 7 days. Therefore, the isolates can be used as promosing candidate for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as tidal lowland and lowland swamp ecosystems in Indonesia.

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Figures and Tables

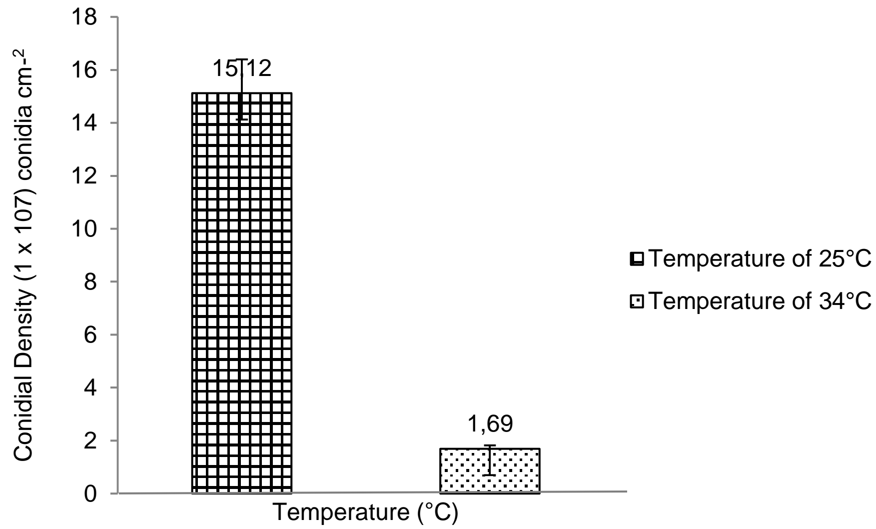


Fig. 1. Conidial density of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C (P = 0.00)

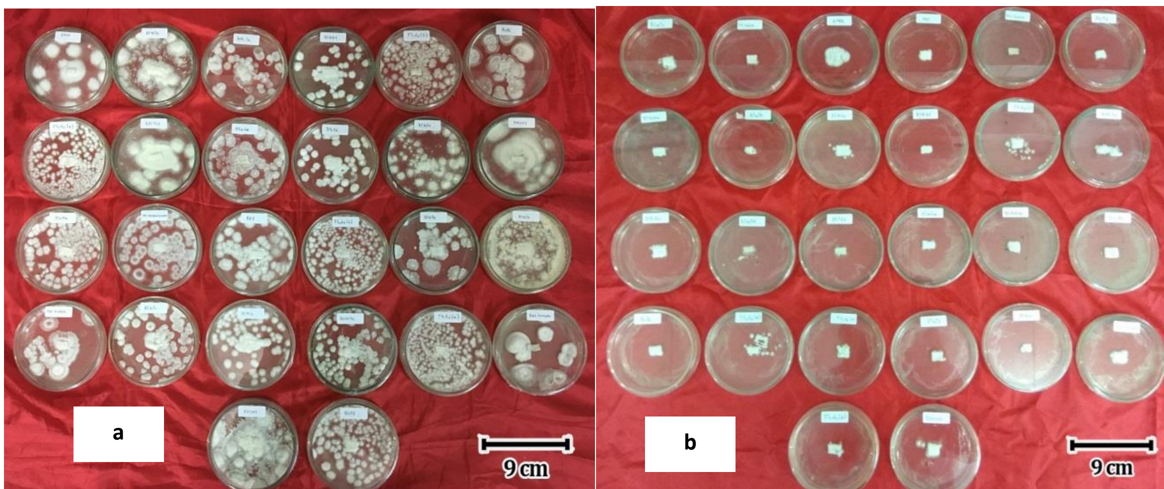


Fig. 2. Colony growth of *Beauveria bassiana* culture incubated for 7 days at 24 °C (a) dan 34 °C (b) (90 mm diameter of petri dish).

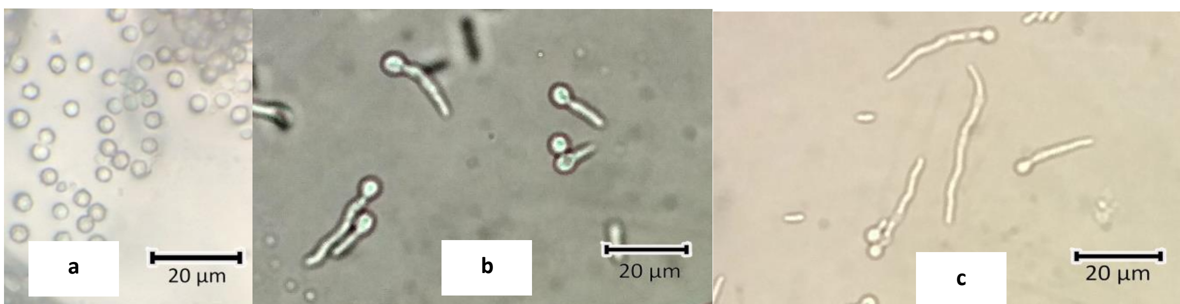
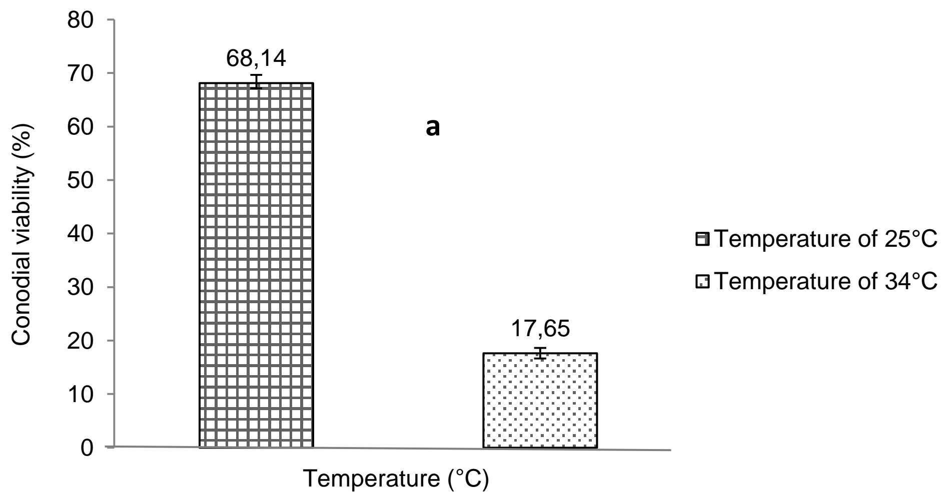


Fig. 3. Conidia of *Beauveria bassiana* (a), viable conidia at 24-hour (b) and 48-hours (c) suspension culture of *Beauveria bassiana* (400x magnification)



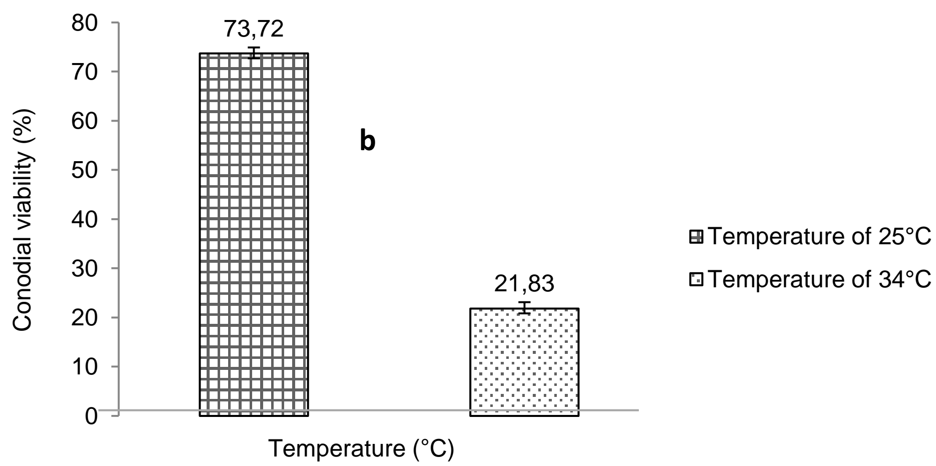


Fig. 4. Conidial viability of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C in 24-hour (P = 0.00) (a) and 48-hour suspension culture (P = 0.00) (b)

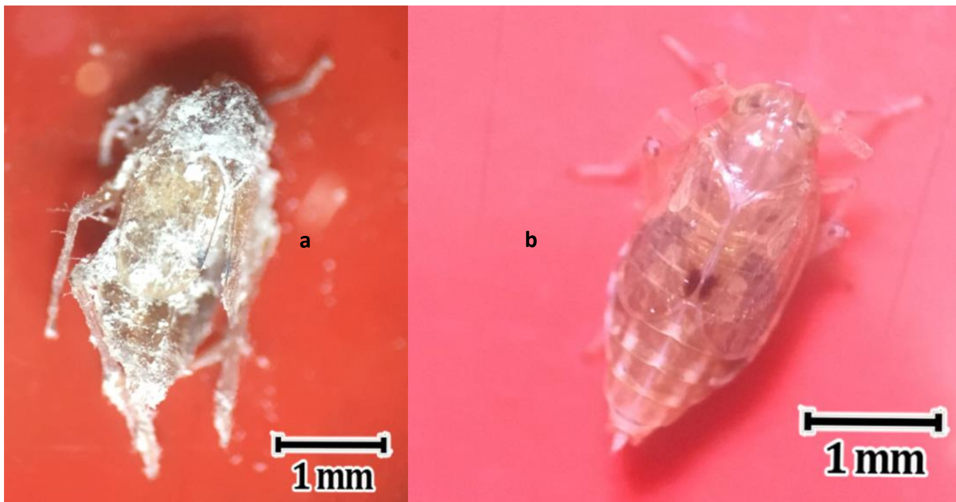


Fig. 5. *Nilaparvata lugens* infected by *Beauveria bassiana* (a) and the healthy one (b)

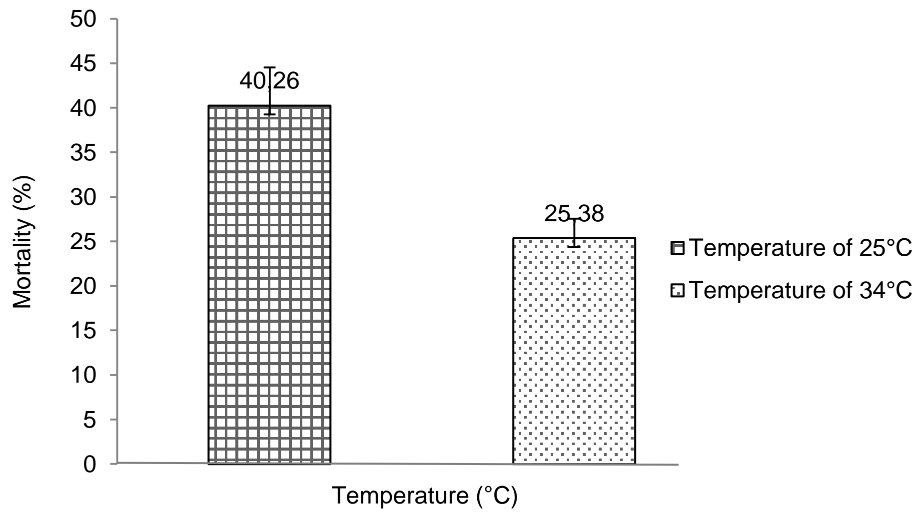
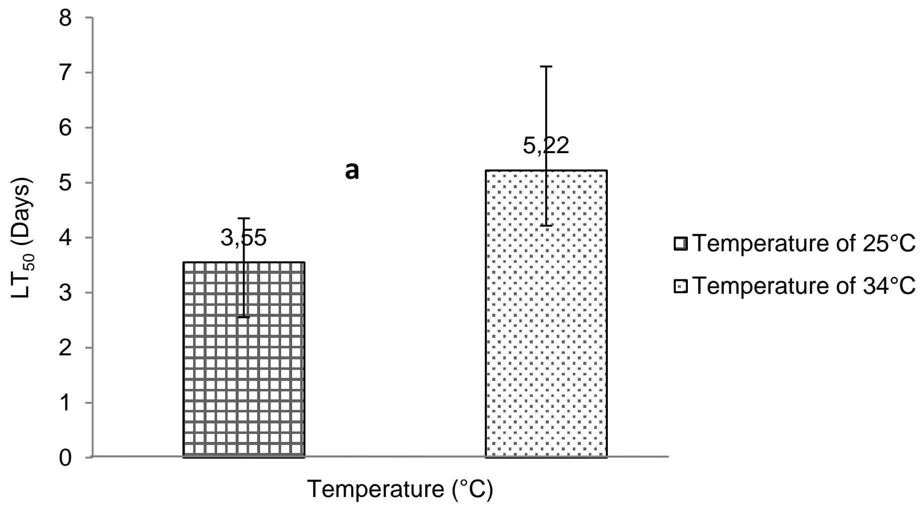


Fig. 6. Mortality of *Nilaparvata lugens* caused by *Beauveria bassiana* culture incubated at 25 and 34 °C (P = 0.003)



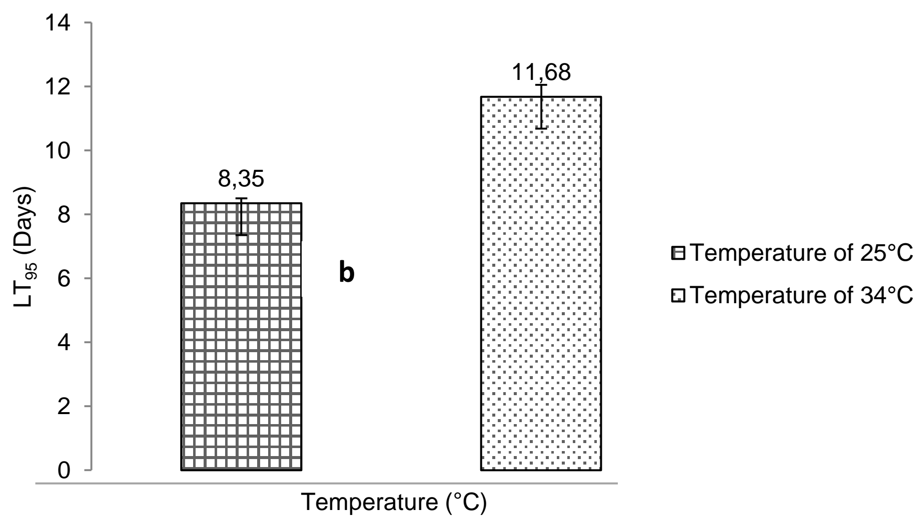


Fig. 7. LT_{50} (P = 0.00) (a) and LT_{95} (P = 0.00) (b) caused by *Beauveria bassiana* culture incubated at 25 and 34 °C against *Nilaparvata lugens*

Table 1. *Beauveria bassiana* isolates used in this research

Isolate codes	Species of fungi	Source (host insects or soil)	Origin (village or city), Province in Indonesia
BPcMs	*Beauveria bassiana*	*Pseudoplusia chalcites*	Muarasiban, South Sumatra
BTmKt	*Beauveria bassiana*	Fresh swamp soils	Kenten, South Sumatra
BTmPc	*Beauveria bassiana*	Fresh swamp soils	Indralaya, South Sumatra
Bws Pantura	*Beauveria bassiana*	*Leptocorisa acuta*	Pantura, West Java
BBY	*Beauveria bassiana*	*Hypothenemus hampei*	Jember, East Java
BTmPe	*Beauveria bassiana*	Fresh swamp soils	Pemulutan, South Sumatra
BTmMa	*Beauveria bassiana*	Fresh swamp soils	Mariana, South Sumatra
BTmSo	*Beauveria bassiana*	Fresh swamp soils	Soak, South Sumatra
BTmSr	*Beauveria bassiana*	Tidal lowland soils	Srikaton, South Sumatra
BuBj	*Beauveria bassiana*	*Alphitobius diaperinu*	Jarai, South Sumatra
725HaJ	*Beauveria bassiana*	*Helopeltis antonii*	Jember, East Java
715HhB	*Beauveria bassiana*	*Hypothenemus hampei*	Banyuwangi, East Java
BTmPd	*Beauveria bassiana*	Highland soils	Pagardin, South Sumatra
BTmTs	*Beauveria bassiana*	Highland soils	Mulia Sari, South Sumatra
BTmkbc	*Beauveria bassiana*	*Chrysodeixis chalcites*	Curup, Bengkulu
BPcPd2	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BPcPd	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BMkMs	*Beauveria bassiana*	Highland soils	Muarasiban, South Sumatra
BTmTr	*Beauveria bassiana*	Tidal lowland soils	Telang Rejo, South Sumatra
Natural BVR[#]	*Beauveria bassiana*	-	-
BTmGa	*Beauveria bassiana*	Fresh swamp soils	Gandus, South Sumatra
BLePd	*Beauveria bassiana*	*Lipaphis erysimi*	Pagardin, South Sumatra
TS1D3A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TSID3B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra

Remarks: [#]Control = commercial products, - unkown source

Table 2. Conidial density of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial density ± SD (10^7 conidia.cm^{-2})	
	25 °C	34 °C
BPcMs	27.34±2.34 m	2.71±0.72 bcd
BTmKt	9.81±0.09 bcde	1.19±0.05 abcd
BTmPc	13.01±0.77 efgh	1.28±0.21 abcd
Bws Pantura	16.87±0.72 hijk	1.61± 0.91 abcd
BBY	19.25±2.49 jkl	1.27±0.24 abcd
BTmPe	12.26±0.48 defg	1.04±0.19 ab
BTmMa	10.93±0.79 cdef	1.15±0.26 abcd
BTmSo	28.21±1.63 m	2.30±0.75 abcd
BTmSr	6.76±0.39 a	1.50±0.66 abcd
BuBj	11.42±0.36 cdef	1.83±0.49 abcd
725HaJ	16.26±3.76 ghij	1.09±0.14 abc
715HhB	7.73±1.43 ab	1.11±0.15 abc
BTmPd	11.35± 0.15 cdef	1.43±0.02 abcd
BTmTs	12.49±1.49 efgh	1.45±0.35 abcd
BTmkbc	14.17±2.44 fghi	1.33±0.39 abcd
BPcPd2	9.40±1.19 bcd	0.97±0.19 a
BPcPd	9.87±1.06 bcde	1.73±0.98 abcd
BMkMs	14.45±0.91 fghi	1.20±0.20 abcd
BTmTr	11.79±1.91 efg	1.24±0.36 abcd
Natural BVR[#]	11.72±0.28 efg	1.55±0.55 abcd

BTmGa	13.07±0.99 efgh	1.54±0.67 abcd
BLePd	8.44±0.25 abc	1.45±0.31 abcd
TS1D3A	21.14±0.58 jklm	3.03±0.79 d
TSID3B	22.82±0.13 klm	2.80±0.24 cd
TS1D2A	28.46±2.64 m	3.00±1.35 cd
TS1D2B	24.02±0.78 lm	3.05±0.87 d
ANOVA F-value	49.05*	3.98*
P value (0.05)	1.7×10^{-37}	1.61×10^{-6}
Tukey's HSD test	0,1383	0.4184

Remarks: * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test, the data were com

Table 3. Conidial viability of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial viability ± SD (%) of 24-hour-suspension culture		Conidial viability ± SD (%) of 48-hour-suspension culture	
	25 °C	34 °C	25 °C	34 °C
BPcMs	70.17±4.80 ab	15.54±1.53 bcdef	77.46±1.59	26.05±2.10 hijk
BTmKt	47.77±9.63 a	16.72±2.27 bcdefg	58.38±2.75	24.75±3.98 fghijk
BTmPc	66.46±7.62 ab	26.30±1.95 hi	76.35±1.97	28.54±1.93 jkl
Bws Pantura	66.51±8.71 ab	9.09±8.32 ab	75.48±13.12	10.57±9.17 ab
BBY	73.02±4.97 ab	23.56±1.3 ghi	75.05±2.88	26.05±2.10 hijk
BTmPe	63.44±12.89 ab	22.28±8.793 fghi	70.85±4.46	25.49±7.21 ghijk
BTmMa	67.94±9.75 ab	14.26±3.90 bcde	72.68±6.60	14.26±3.91 bcd
BTmSo	74.83±6.15 ab	19.71±3.78 cdefgh	77.55±6.13	22.47±1.86 fghi
BTmSr	73.62±8.85 ab	20.99±1.89 defgh	75.47±7.67	20.99±1.89 efgh
BuBj	74.30±6.82 ab	21.64±4.26 efghi	78.69±4.45	22.92±2.76 fghij
725HaJ	66.49±10.80 ab	14.03±2.93 bcd	72.10±7.62	16.63±5.18 cde
715HhB	68.77±2.28 ab	11.20±9.71 abc	74.43±4.34	13.32±11.73 bc
BTmPd	75.19±10.56 ab	23.77±4.46 ghi	76.45±9.77	28.52±2.66 jkl
BTmTs	76.30±11.60 ab	12.40±3.79 abc	79.85±10.47	12.40±3.79 bc
BTmkbc	66.24±1.30 ab	22.51±4.88 fghi	70.43±1.53	22.51±4.88 fghi
BPcPd2	71.44±8.88 ab	7.98±7.19 a	70.42±3.09	7.98±7.19 a
BPcPd	63.60±18.14 ab	15.10±3.93 bcdef	69.81±12.77	15.10±3.93 bcd
BMkMs	65.06±5.80 ab	14.42±6.65 bcde	72.14±6.04	21.53±0.67 efgh
BTmTr	70.00±13.82 ab	15.44±1.84 bcdef	73.70±7.60	24.02±2.00 ghijk
Natural BVR#	60.74±9.62 ab	28.53±1.06 i	69.73±10.85	32.58±3.49 l
BTmGa	63.42±11.63 ab	19.89±1.29 cdefgh	67.15±8.12	19.89±1.29 defg
BLePd	48.80±6.83 a	17.17±3.87 cdefg	59.00±4.84	19.31±3.44 def

TS1D3A	79.33±6.36 b	17.75±3.90 cdefg	81.42±4.73	25.61±1.77 ghijk
TSID3B	69.49±4.96 ab	15.87±1.02 bcdef	80.23±5.33	28.72±1.60 jkl
TS1D2A	78.41±4.33 b	17.69±0.92 cdefg	81.38±1.38	28.12±5.33 ijkl
TS1D2B	70.29±4.45 ab	15.14±4.07 bcdef	80.58±7.39	29.22±4.32 kl
ANOVA F-value	2.02*	3.40*	2.03ns	5.89*
P value (0.05)	10×10^{-8}	4.6×10^{-6}	$9,7 \times 10^{-10}$	9×10^{-10}
Tukey's HSD test	18,057	7.45	-	7.56

Remarks: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at $P < 0.05$ according to HSD test

Table 4. Virulence of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C against *Nilaparvata lugens* nymphs

Isolate codes	Mortality of *Nilaparvata lugens* nymphs ± SD (%)	
	25 °C	**34 °C**
BPcMs	65.00±5.00	21.67±5.77
BTmKt	26.67±10.41	13.33±14.43
BTmPc	43.33±7.64	28.33±7.64
Bws Pantura	30.00±18.03	5.00±5.00
BBY	38.33±15.28	16.67±7.64
BTmPe	28.33±10.41	21.67±17.56
BTmMa	28.33±14.43	25.00±18.03
BTmSo	56.67±10.41	40.00±18.03
BTmSr	35.00±5.00	35.00±5.00
BuBj	45.00±21.79	26.67±28.87
725HaJ	23.33±2.89	20.00±5.00
715HhB	15.00±5.00	6.67±7.64
BTmPd	26.67±7.64	20.00±15.00
BTmTs	26.67±10.41	13.33±2.89
BTmkbc	28.33±15.28	28.33±15.28
BPcPd2	20.00±10.00	10.00±10.00
BPcPd	25.00±10.00	25.00±10.00
BMkMs	26.67±14.43	26.67±14.43
BTmTr	31.67±24.66	20.00±5.00

Natural BVR#	43.33±12.58	36.67±7.64
BTmGa	30.00±5.00	30.00±5.00
BLePd	26.67±11.55	23.33±5.77
TS1D3A	90.00±13.23	38.33±11.55
TSID3B	58.33±7.64	43.33±14.43
TS1D2A	81.67±20.21	41.67±15.28
TS1D2B	96.67±5.77	43.33±18.93
ANOVA F-value	0.29ns	0.16ns
P value (0.05)	0.99	1.00

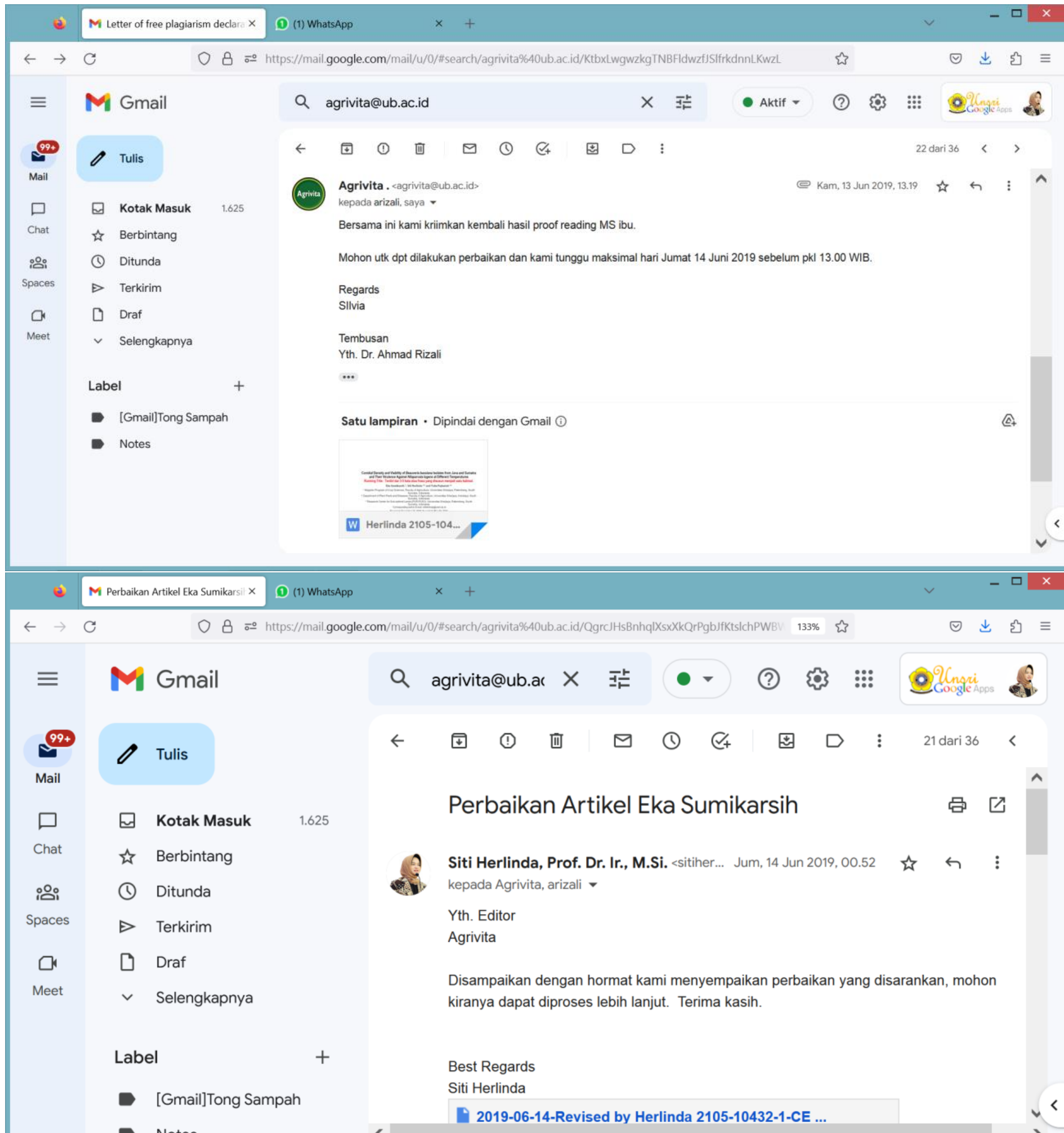
Remarks: ns = not significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test

Table 5. LT_{50} and LT_{95} of *Nilaparvata lugens* nymphs caused by *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	LT_{50} (days) (95% fiducial limits)		LT_{95} (days) (95% fiducial limits)	
	25 °C	34 °C	25 °C	34 °C
BPcMs	2.62 (1.68 – 3.72)	4.95 (3.71 – 6.14)	7.43 (5.66 – 9.15)	11.40 (9.43 – 12.78)
BTmKt	4.18 (3.37 – 4.82)	7.48 (5.77 – 8.76)	9.00 (8.35 – 10.25)	13.93 (11.63 – 16.56)
BTmPc	3.86 (2.58 – 5.28)	4.87 (4.26 – 5.20)	8.58 (7.71 – 10.03)	11.33 (9.97 – 13.00)
Bws Pantura	4.31 (2.86 – 6.85)	10.40 (7.22 – 14.22)	9.13 (8.26 – 10.83)	16.85 (12.94 – 22.01)
BBY	2.93 (2.60 – 3.33)	5.96 (3.62 – 8.12)	7.75 (6.84 – 8.76)	12.41 (9.34 – 15.91)
BTmPe	3.88 (3.65 – 4.23)	5.92 (3.11 – 10.56)	8.69 (8.21 – 9.08)	12.37 (8.96 – 18.36)
BTmMa	2.76 (2.01 – 4.16)	2.99 (1.36 – 4.42)	7.57 (6.09 – 9.19)	9.45 (7.08 – 12.22)
BTmSo	3.31 (2.68 – 4.42)	3.09 (2.56 – 3.68)	8.13 (7.71 – 8.40)	9.55 (8.27 -11.48)
BTmSr	2.57 (1.94 – 2.95)	2.92 (2.40 – 3.60)	7.39 (6.94 – 8.26)	9.38 (8.11 – 10.56)
BuBj	2.68 (1.76 – 4.16)	6.43 (1.45 – 10.35)	7.49 (6.09 – 9.19)	12.89 (7.17 – 18.14)
725HaJ	3.19 (2.73 – 3.76)	5.65 (4.35 – 7.04)	8.00 (7.05 – 8.79)	12.00 (11.41 – 12.45)
715HhB	5.06 (2.98 – 6.76)	9.00 (6.92 – 10.47)	9.88 (6.96 – 12.19)	15.46 (12.77 – 17.42)
BTmPd	2.24 (1.59 – 3.24)	5.02 (2.73 – 8.85)	7.06 (5.88 – 8.67)	11.47 (8.44 – 16.65)
BTmTs	3.93 (3.07 – 4.98)	6.55 (5.44 – 8.12)	8.75 (7.05 – 10.40)	13.01 (11.15 – 15.91)
BTmkbc	4.61 (3.24 – 5.32)	3.31 (1.21 – 6.13)	9.13 (7.77 – 10.31)	9.76 (6.92 – 13.93)
BPcPd2	4.21 (2.73 – 6.82)	8.15 (2.75 – 14.22)	9.02 (7.05 – 11.85)	14.61 (8.46 – 22.01)
BPcPd	4.39 (3.09 – 5.65)	4.37 (3.09 – 6.51)	9.20 (8.40 – 10.68)	10.91 (8.80 – 12.64)
BMkMs	4.19 (2.66 – 7.09)	3.95 (2.23 – 6.65)	9.01 (6.64 – 12.12)	10.74 (7.95 – 12.51)
BTmTr	3.82 (2.91 – 4.65)	4.66 (3.97 – 5.09)	8.64 (6.89 – 10.08)	11.12 (9.82 – 12.72)
Natural BVR#	4.12 (3.37 – 5.44)	4.06 (3.65 – 4.65)	8.94 (7.35 – 10.47)	10.51 (9.71 – 11.45)
BTmGa	3.68 (2.93 – 4.36)	3.96 (2.94 – 4.66)	8.50 (8.35 – 8.79)	10.41 (8.66 – 12.08)
BLePd	3.90 (3.33 – 4.59)	3.79 (3.50 – 3.97)	8.72 (8.36 – 9.22)	10.25 (9.62 – 11.29)
TS1D3A	2.99 (2.46 – 3.55)	4.17 (4.07 – 4.32)	7.80 (6.94 – 8.98)	10.63 (9.79 – 11.92)
TSID3B	2.34 (1.63 – 3.72)	3.92 (3.23 – 4.91)	7.16 (5.61 – 9.15)	10.38 (9.48 – 11.02)
TS1D2A	2.41 (1.19 – 3.10)	5.80 (4.17 – 7.26)	7.23 (6.62 – 7.97)	12.26 (9.88 – 13.78)
TS1D2B	4.19 (3.05 – 5.28)	4.35 (3.48 – 4.82)	9.01 (8.23 – 10.31)	10.67 (10.13 -11.28)
ANOVA F-value	0.84ns	2.08ns	0.84ns	1.21ns
P value (0.05)	0.68	0.01	0.68	0.28

Remarks: ns = not significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test

3. Bukti konfirmasi review kedua dan hasil revisi kedua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Disampaikan dengan hormat kami menyempaikan perbaikan yang disarankan, mohon kiranya dapat diproses lebih lanjut. Terima kasih.

Best Regards
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Conidial Density and Viability of *Beauveria bassiana* Isolates from Java and Sumatra and Their Virulence Against *Nilaparvata lugens* at Different Temperatures

Running Title: *Beauveria bassiana* for *Nilaparvata lugens*

Eka Sumikarsih [1], Siti Herlinda [2,3*] and Yulia Pujiastuti [2,3]

[1] Magister Program of Crop Sciences, Faculty of Agriculture, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia
[2] Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, South Sumatra, Indonesia
[3] Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang, South Sumatra, Indonesia
[*] Corresponding author E-mail: sitiherlinda@unsri.ac.id

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ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* can cause direct damage and transmit rice diseases. *Beauveria bassiana* is used to control BPH, however the success of the fungal efficacy on rice fields is affected by external factors, such as temperature. This research aimed to evaluate the conidial viability and density of *B. bassiana* isolates from Java and Sumatra, exposed to 25 and 34°C and their virulence against BPH nymphs. Twenty six isolates of *B. bassiana* cultures incubated at 25 and 34°C for 7 days were observed on their conidial density, viability, and virulence against BPH nymphs. The incubation temperature of 34°C was able to decrease conidial density and viability, and virulence of the isolates. However, some isolates of *B. bassiana* originated from soils or insects in Sumatra, especially from South Sumatra still produced high conidial density and viability as well as high virulent against BPH nymphs, such as TS1D3A, TSID3B, TS1D2A and TS1D2B isolates. The TS1D2B isolate incubated at 34°C still caused the highest percentage of BPH mortality (43.33%) among other isolates. Therefore, the isolates can be used as promosing candidate for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as wetland or lowland rice ecosystems in Indonesia.

Keywords: biocontrol; brown planthopper; entomopathogenic fungus; mortality

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae) is the most serious insect pest which sucks phloem sap on stems of rice (*Oryza sativa* L.) (Daravath & Chander, 2017; Herlinda et al., 2018). A direct damage caused by this BPH results in hampered growth of rice producing 'hopperburn' (Dharshini & Siddegowda, 2015). In addition, BPH can also as an insect vector that transmits rice diseases, such as the ragged stunt and grassy stunt virus (Dietzgen, Mann, & Johnson, 2016; Zheng, Mao, Xie, & Wei, 2014). The attack of BPH do not only occure in Indonesia, but also attack rice in several Asian countries, such as China, Vietnam, Thailand, India, Pakistan, Malaysia and Philippine (Catiding et al., 2009; Hu et al., 2014). BPH also attacked rice in Texas (Leavengood, Bartlett, & Vitanza-Hedman, 2017).

The effort to control population of *N. lugens* had been done through synthetic chemical control (Baehaki & Suparno, 2018; Liu et al., 2013; Zhang et al., 2014) and biological control by using entomopathogenic fungi, such as *Beauveria bassiana* (Lee et al., 2015; Li, Lin, Li, Xu, & Feng, 2012) and *Metarhizium anisopliae* (Chinniah, Ravikumar, Kalyanasundaram, & Parthiban, 2016) have been proven to be effective agents to control the BPH. *B. bassiana* could kill BPH more than 80 % (Lee et al., 2015; Li et al., 2014) and to kill the eggs of BPH as well as to disturb adult stage proliferation (Li, Lin, Li, Xu, & Feng, 2012). *B. bassiana* is also not harmful toward natural enemies of the BPH (Firouzbakht, Zibaee, Hoda, & Sohani, 2015; Gholamzadeh-Chitgar, Hajizadeh, Ghadamyari, Karimi-Malati, & Hoda, 2017).

Although *B. bassiana* had proven to be effective in controlling the BPH, the success of its efficacy on rice fields was affected by many external factors, such as temperature (Ghany, 2015). Extremely high temperature can results in death of the fungus (Ottati-de-Lima et al., 2014). The optimum temperature for growth of the entomopathogenic fungi usually is in the range of 25 to 30 ºC (Bugeme, Maniania, Knapp, & Boga, 2008). Most of entomopathogenic fungi tolerate to temperatures in the range of 0 to 40 °C (Ghany, 2015), but certain strains of entomopathogenic fungi can only survive at temperatures below 35 °C (Constanski et al., 2011). The production of colony and conidial density of *B. bassiana* is significantly decreased if the temperature during fungal incubation increases from 30 to 35 °C (Ottati-de-Lima et al., 2014) and all isolates are dead at temperature of 36 °C (Ottati-de-Lima et al., 2014; Pham, Kim, Kim, & Kim, 2009). Fungal germination is also decrease at temperature above 30 °C (Pham, Kim, Kim, & Kim, 2009) with the highest level up to 33 °C (Salim, Md. Rawi, Ahmad, & Al-Shami, 2015), whereas temperature of 25 °C is an ideal temperature for the fungal germination (Lohse, Jakobs-Schönwandt, & Patel, 2014). Virulence of the entomopathogenic fungi can be affected by temperature (Bugeme, Maniania, Knapp, & Boga, 2008; Constanski et al., 2011; Ghany, 2015; Ottati-de-Lima et al., 2014; Satpathi, Acharjee, & Saha, 2016; Tefera & Pringle, 2003). Each strain/isolate or species of the entomopathogenic fungi also has different optimum temperature and tolerance level to temperature. Entomopathogenic fungal strains that can survive at extremely high temperature of above 33 °C are superior strains (Salim, Md. Rawi, Ahmad, & Al-Shami, 2015). These superior strains can be used as candidates to control the BPH in tropical ecosystems, such as agroecosystems in Indonesia. Therefore, the objectives of this research were to evaluate the conidial viability and density of *B. bassiana* isolates form Java and Sumatra exposed to 25 and 34 °C temperatures and their virulence against *N. lugens* nymphs.

MATERIALS AND METHODS

Study Site

This research was conducted at Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, from September 2017 to April 2018. Isolates used in

this research were isolates that collected from soil of lowland swamps, tidal lowlands, peatlands, and highlands in South Sumatra, whereas isolates from soil and infected insects obtaining from other provinces used as comparison as well as one commercial isolate as control (Table 1). The species of the fungus was identified by Dr. Suwandi, a microbiologist from Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya. The average room temperature during the experiment was 30.5 °C and the average relative humidity was 84.30 % within the laboratory during the experiment.

Preparation of Test Insects

Adults and nymphs of N. lugens were collected from fields of rice at Indralaya, South Sumatra from September 2017 to March 2018 and brought to the Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya for identification. Then, the nymph and adults were reared and maintained on 5 clumps of 10-day-old rice grown within bucket (bottom diameter of 20 cm, upper diameter of 25 cm, and height of 20 cm) within a greenhouse at temperature range of 30 to 35 °C. The rice was put in wire mesh enclosure (height of 100 cm, length of 50 cm, and width of 50 cm). Within one of wire mesh enclosure, 10 pairs of the adults were released in order to infect the rice for 10 days and subsequently the infected rice crops were substituted with the healthy one and this propagation was done on 30 cages. Fresh and healthy rice were given to newly emerged nymphs of N. lugens and this was maintained up to at least five generations. The sixth generation and henceforth generations were used and selected for the fourth instar for bioassay in this experiment.

Isolates Preparation of *Beauveria bassiana*

All isolates used in this study were previously fitted by using living insects of *Tenebrio molitor.* Fresh cultures of *B. bassiana* were started by inoculating Sabouraud Dextrose Agar (SDA) (Oxoid) with the third larvae of *T. molitor*. Prior to inoculation, *B. bassiana* and *T. molitor* were previously sterilized by using modified method from Nuraini, Setyaningsih, & Susilowati (2017). Subsequently, the fungal culture was incubated for 7 x 24 hours in order to produce sufficients numbers of fungal colony. The *B. bassiana* culture that had previously fitted was then used for subsequent observation. The fresh *B. bassiana* culture was cut with dimension of 10 mm x 10 mm for recultured into SDA medium which would be used for subsequent observation consisting of conidial density and viability as well as virulence test.

Observation of Conidial Density and Viability

Twenty six isolates of the *B. bassiana* culture were grown on SDA medium and then each isolate was incubated at constant temperature of 25 and 34 °C within incubator for 7 x 24 hours using three replications. The ideal temperature of 25 °C and extremely high temperature of 34 °C in this research were chosen for culturing of *B. bassiana* for 7 days. Both temperature were chosen because the temperature of 25 °C is ideal temperature for culture *B. bassiana* (Bugeme, Maniania, Knapp, & Boga, 2008), whereas temperature higher than 33 °C is extremely high temperature (Salim, Md. Rawi, Ahmad, & Al-Shami, 2015). Conidia of all isolates at the 8th day were counted in term of their density of the 7 day *B. bassiana* culture. Calculation of conidial density was started with fungal suspension production by harvesting 10 mm x 10 mm (1 cm^2) 7-day *B. bassiana* culture which followed by 10 ml addition of sterile distilled water. The suspension was vortexed using turbo mixer for 20 seconds in order to produce homogenous conidial suspension. This suspension culture was diluted through addition of 9 ml sterile distilled water into 1 ml *B. bassiana* suspension culture, homogenized. Subsequently, the last suspension culture was counted in term of its conidial density under a compound microscope at 400x magnification that had been equipped with haemocytometer. This treatments were arranged in completely randomized design and replicated three times.

Table 1. *Beauveria bassiana* isolates used in this research

Isolate codes	Species of fungi	Source (host insects or soil)	Origin (village or city), Province in Indonesia
BPcMs	*Beauveria bassiana*	*Pseudoplusia chalcites*	Muarasiban, South Sumatra
BTmKt	*Beauveria bassiana*	Fresh swamp soils	Kenten, South Sumatra
BTmPc	*Beauveria bassiana*	Fresh swamp soils	Indralaya, South Sumatra
Bws Pantura	*Beauveria bassiana*	*Leptocorisa acuta*	Pantura, West Java
BBY	*Beauveria bassiana*	*Hypothenemus hampei*	Jember, East Java
BTmPe	*Beauveria bassiana*	Fresh swamp soils	Pemulutan, South Sumatra
BTmMa	*Beauveria bassiana*	Fresh swamp soils	Mariana, South Sumatra
BTmSo	*Beauveria bassiana*	Fresh swamp soils	Soak, South Sumatra
BTmSr	*Beauveria bassiana*	Tidal lowland soils	Srikaton, South Sumatra
BuBj	*Beauveria bassiana*	*Alphitobius diaperinu*	Jarai, South Sumatra
725HaJ	*Beauveria bassiana*	*Helopeltis antonii*	Jember, East Java
715HhB	*Beauveria bassiana*	*Hypothenemus hampei*	Banyuwangi, East Java
BTmPd	*Beauveria bassiana*	Highland soils	Pagardin, South Sumatra
BTmTs	*Beauveria bassiana*	Highland soils	Mulia Sari, South Sumatra

BTmkbc	*Beauveria bassiana*	*Chrysodeixis chalcites*	Curup, Bengkulu
BPcPd2	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BPcPd	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BMkMs	*Beauveria bassiana*	Highland soils	Muarasiban, South Sumatra
BTmTr	*Beauveria bassiana*	Tidal lowland soils	Telang Rejo, South Sumatra
Natural BVR#	*Beauveria bassiana*	-	-
BTmGa	*Beauveria bassiana*	Fresh swamp soils	Gandus, South Sumatra
BLePd	*Beauveria bassiana*	*Lipaphis erysimi*	Pagardin, South Sumatra
TS1D3A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TSID3B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra

Remarks: #Control = commercial products, - unkown source

Table 2. Conidial density of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial density ± SD (10^7 conidia per cm^2)	
	25 °C	34 °C
BPcMs	27.34±2.34 m	2.71±0.72 bcd
BTmKt	9.81±0.09 bcde	1.19±0.05 abcd
BTmPc	13.01±0.77 efgh	1.28±0.21 abcd
Bws Pantura	16.87±0.72 hijk	1.61± 0.91 abcd
BBY	19.25±2.49 jkl	1.27±0.24 abcd
BTmPe	12.26±0.48 defg	1.04±0.19 ab
BTmMa	10.93±0.79 cdef	1.15±0.26 abcd
BTmSo	28.21±1.63 m	2.30±0.75 abcd
BTmSr	6.76±0.39 a	1.50±0.66 abcd
BuBj	11.42±0.36 cdef	1.83±0.49 abcd
725HaJ	16.26±3.76 ghij	1.09±0.14 abc
715HhB	7.73±1.43 ab	1.11±0.15 abc
BTmPd	11.35± 0.15 cdef	1.43±0.02 abcd
BTmTs	12.49±1.49 efgh	1.45±0.35 abcd
BTmkbc	14.17±2.44 fghi	1.33±0.39 abcd
BPcPd2	9.40±1.19 bcd	0.97±0.19 a
BPcPd	9.87±1.06 bcde	1.73±0.98 abcd
BMkMs	14.45±0.91 fghi	1.20±0.20 abcd
BTmTr	11.79±1.91 efg	1.24±0.36 abcd
Natural BVR#	11.72±0.28 efg	1.55±0.55 abcd
BTmGa	13.07±0.99 efgh	1.54±0.67 abcd
BLePd	8.44±0.25 abc	1.45±0.31 abcd
TS1D3A	21.14±0.58 jklm	3.03±0.79 d
TSID3B	22.82±0.13 klm	2.80±0.24 cd
TS1D2A	28.46±2.64 m	3.00±1.35 cd
TS1D2B	24.02±0.78 lm	3.05±0.87 d
ANOVA F-value	49.05*	3.98*
P value (0.05)	1.7×10^{-37}	1.61×10^{-6}
Tukey's HSD test	0.1383	0.4184

Remarks: * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at $P < 0.05$ according to HSD test

The calculation of conidial viability was based on percentage of conidial germination. The conidial germination was observed from the 7-day *B. bassiana* culture which incubated at 25 or 34 °C and grown on one layer of SDA medium according to the method of Bugeme, Maniania, Knapp, & Boga (2008). The 7-day *B. bassiana* suspension culture was scratched with magnitude of 0.1 ml on SDA plates. A sterile microscope cover slip was placed on each plate, then each suspension culture was incubated for 24 and 48 hours at temperature of 25 °C. Furthermore, the germinated conidia was observed under microscope and calculation was done in term of numbers of germinated conidia. The germination percentage was determined from 100-spores for each plate using the compound microscope. These treatments were arranged in completely randomized design and replicated three times.

Bioassay Procedure

Bioassays by modification of Trizelia & Nurdin (2010) method were conducted to determine the virulence of *B. bassiana* isolates againts *N. lugens* nymphs. The 26 isolates of *B. bassiana* were incubated at 25 and 34 °C for 7 days and their conidia were harvested. Each isolates of *B. bassiana* was topically sprayed with 10 ml of a concentration of 1 x 10^3 conidia per cm^2 on 25 fourth-nymphs of *N. lugens* placed on a filter paper in petri dishes. Then, the nymphs of *N. lugens* were placed into 20 healthy two-weeks rice stems. This treatments were arranged in completely randomized design and replicated three times. Numbers of dead *N. lugens* nymphs was recorded every 12 hours for 11 days period which used to determine the percentage of mortality and lethal time to 50 % (LT_{50}) and 95 % (LT_{95}) mortality of *N. lugens* nymphs. The behaviour change of *N. lugens* infested by *B. bassiana* was observed and recorded daily until the insects were dead and mycelia cover all of their bodies. The dead nymphs were transferred into petri dishes lined with moist-sterile filter paper to allow the growth of the *B. bassiana* on the surface of the cadaver.

Data Analysis

Data of conidial density and viability, and percentage of mortality among treatments were analyzed by using analysis of variance (ANOVA). If there were differences among the data of each isolate, then Honestly Significant Different (HSD) test at 5 % was conducted by using program software of SAS University Edition 2.7 9.4 M5. The data between temperature were compared using t test. LT_{50} and LT_{95} values were calculated using probit analysis.

RESULTS AND DISCUSSION

Conidial Density and Viability of *Beauveria bassiana*

B. bassiana culture that was incubated for 7 days at temperature of 25 °C showed the highest conidial density on BTmSo isolate and was not significantly different from that of BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B isolates (Table 2). At incubation temperature of 34 °C, the highest value of conidial density was found on TS1D2B isolate and was significantly different than that of BPcPd2 isolate which had the lowest conidial density value. Conidial density of all isolates at 25 °C were siginificantly higher than the isolates at 34 °C (P = 0.00) (Fig. 1). Conidial density significantly decreased with the increase of the fungal incubation temperature and followed by more hampered of fungal colony growth. Fungal culture incubated for 7 days at 25 °C had normal growth with colony diameter in the range of 50 to 90 mm, whereas colony of *B. bassiana* incubated at 34 °C only had diameter in the range of 15 to 30 mm (Fig. 2). Incubation temperature of 34 °C for *B. bassiana* culture was able to decrease conidial density and colony growth of the fungus; however some isolates (TS1D3A, TSID3B, TS1D2A and TS1D2B isolates) still achieved high conidial density and colony growth.

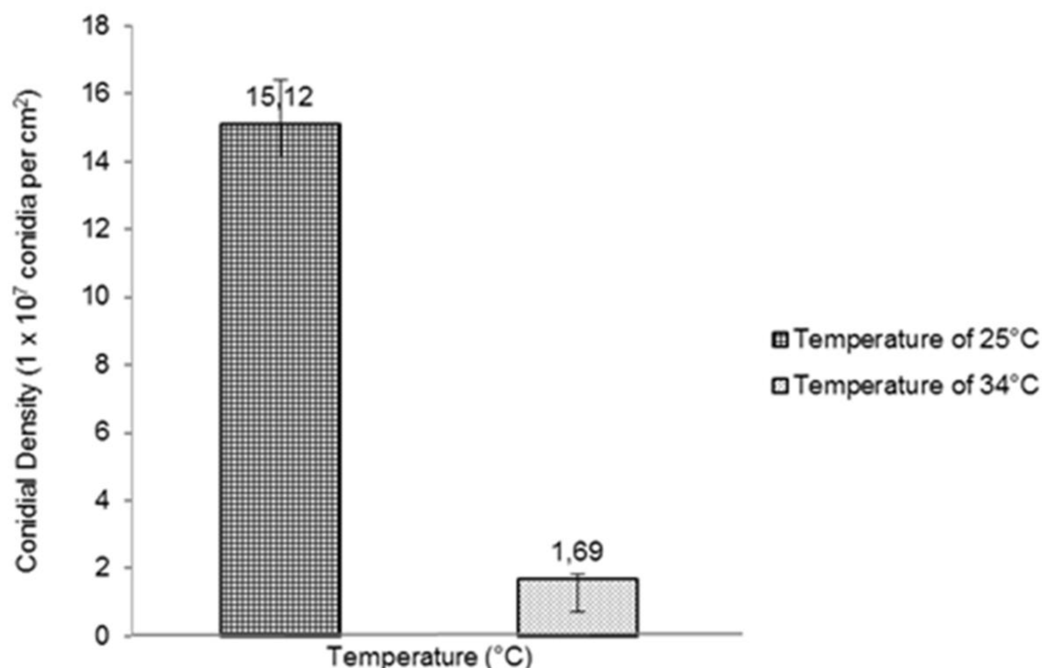

Fig. 1. Conidial density of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C (P = 0.00)

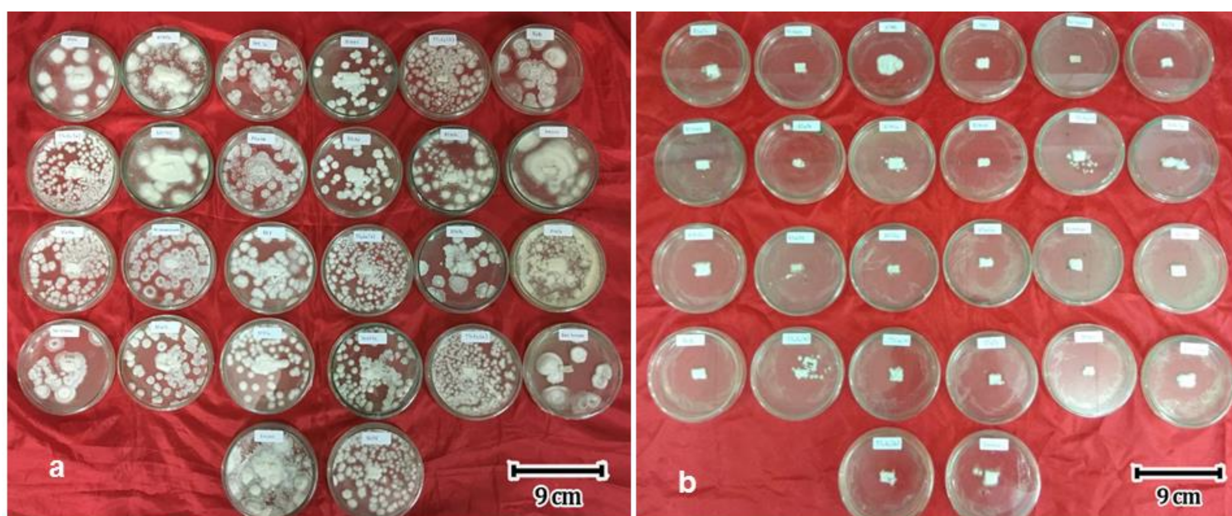

Fig. 2. Colony growth of *Beauveria bassiana* culture incubated for 7 days at 24 °C (a) and 34 °C (b) (90 mm diameter of petri dish).

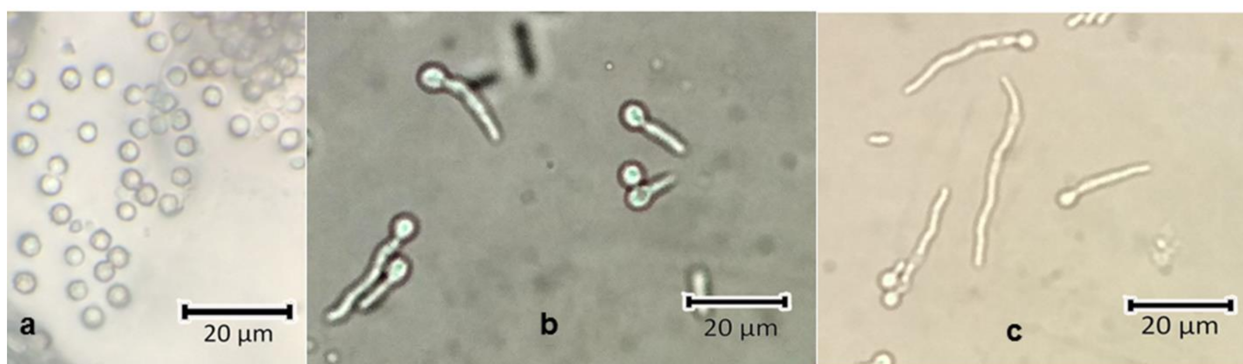

Fig. 3. Conidia of *Beauveria bassiana* (a), viable conidia at 24-hour (b) and 48-hours (c) suspension culture of *Beauveria bassiana* (400x magnification)

Conidial density values of *B. bassiana* isolate cultures incubated at 25 °C were all high, but higher values were found on isolates of BtmSo, BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B. They were isolates from South Sumatra. Conidial density of all isolates were siginificantly decrease if the isolates were incubated at 34 °C. However, only TS1D2B isolate still had high conidial density at incubation temperature of 34 °C. The conidial density decreased at 34 °C due to the lower production of conidia per cm^2 in agar medium which indicated by hampered colony growth of *B. bassiana* (Fig. 2). The diameter of colony growth reached 90 mm at 25 °C, whereas the diameter of colony growth at 34 °C was only 15-30 mm. Ottati-de-Lima et al. (2014) had stated that *M. anisopliae* could yield optimal colonies in liquid medium from 25 to 30 °C and temperature of 35 °C was detrimental to colony growth of the fungus. High spore or conidial production of *B. bassiana* occurred at 25-27 °C (Pham, Kim, Kim, & Kim, 2009). The incubation temperature of 34 °C in this research could decrease the conidial density and colony growth of *B. bassiana*.

Table 3. Conidial viability of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial viability ± SD (%) of 24-hour-suspension culture		Conidial viability ± SD (%) of 48-hour-suspension culture	
	25 °C	34 °C	25 °C	34 °C
BPcMs	70.17±4.80 ab	15.54±1.53 bcdef	77.46±1.59	26.05±2.10 hijk
BTmKt	47.77±9.63 a	16.72±2.27 bcdefg	58.38±2.75	24.75±3.98 fghijk
BTmPc	66.46±7.62 ab	26.30±1.95 hi	76.35±1.97	28.54±1.93 jkl
Bws Pantura	66.51±8.71 ab	9.09±8.32 ab	75.48±13.12	10.57±9.17 ab

BBY	73.02±4.97 ab	23.56±1.3 ghi	75.05±2.88	26.05±2.10 hijk
BTmPe	63.44±12.89 ab	22.28±8.793 fghi	70.85±4.46	25.49±7.21 ghijk
BTmMa	67.94±9.75 ab	14.26±3.90 bcde	72.68±6.60	14.26±3.91 bcd
BTmSo	74.83±6.15 ab	19.71±3.78 cdefgh	77.55±6.13	22.47±1.86 fghi
BTmSr	73.62±8.85 ab	20.99±1.89 defgh	75.47±7.67	20.99±1.89 efgh
BuBj	74.30±6.82 ab	21.64±4.26 efghi	78.69±4.45	22.92±2.76 fghij
725HaJ	66.49±10.80 ab	14.03±2.93 bcd	72.10±7.62	16.63±5.18 cde
715HhB	68.77±2.28 ab	11.20±9.71 abc	74.43±4.34	13.32±11.73 bc
BTmPd	75.19±10.56 ab	23.77±4.46 ghi	76.45±9.77	28.52±2.66 jkl
BTmTs	76.30±11.60 ab	12.40±3.79 abc	79.85±10.47	12.40±3.79 bc
BTmkbc	66.24±1.30 ab	22.51±4.88 fghi	70.43±1.53	22.51±4.88 fghi
BPcPd2	71.44±8.88 ab	7.98±7.19 a	70.42±3.09	7.98±7.19 a
BPcPd	63.60±18.14 ab	15.10±3.93 bcdef	69.81±12.77	15.10±3.93 bcd
BMkMs	65.06±5.80 ab	14.42±6.65 bcde	72.14±6.04	21.53±0.67 efgh
BTmTr	70.00±13.82 ab	15.44±1.84 bcdef	73.70±7.60	24.02±2.00 ghijk
Natural BVR#	60.74±9.62 ab	28.53±1.06 i	69.73±10.85	32.58±3.49 l
BTmGa	63.42±11.63 ab	19.89±1.29 cdefgh	67.15±8.12	19.89±1.29 defg
BLePd	48.80±6.83 a	17.17±3.87 cdefg	59.00±4.84	19.31±3.44 def
TS1D3A	79.33±6.36 b	17.75±3.90 cdefg	81.42±4.73	25.61±1.77 ghijk
TSID3B	69.49±4.96 ab	15.87±1.02 bcdef	80.23±5.33	28.72±1.60 jkl
TS1D2A	78.41±4.33 b	17.69±0.92 cdefg	81.38±1.38	28.12±5.33 ijkl
TS1D2B	70.29±4.45 ab	15.14±4.07 bcdef	80.58±7.39	29.22±4.32 kl
ANOVA F-value	2.02*	3.40*	2.03ns	5.89*
P value (0.05)	10 x 10^{-8}	4.6x10^{-6}	9.7 x 10^{-10}	9x10^{-10}
Tukey's HSD test	18.057	7.45	-	7.56

Remarks: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test

Viable or germinate conidia was characterized by the following aspects: the change of size and form of conidia compared to size and form of normal conidia (Fig. 3a), conidial wall broke and produced germ tube and followed by elongation of the germ tube (Fig. 3b and 3c). Percentage of conidial germination was used to determine conidial viability. At incubation temperature of 25 °C, the highest value of conidial viability of 24-hour-suspension culture was found on TS1D3A isolate and was not significantly different from other isolates, except BTmKt and BLePd isolates (Table 3). However, the highest value of conidial viability at temperature of 34 °C was found on Natural BVR# isolate in control of commercial product and was not significantly different from other five isolates consisting of BTmPc, BBY, BtmPe, BTmPd and BTmkbc. Conidial viability of 48-hour-suspension culture at 25 °C for all isolates were high and not significantly different among isolates. Nevertheless, isolates that had the highest conidial viability at 34 °C was Natural BVR# isolate and was not significantly different from BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B isolates. The temperature increase during incubation of *B. bassiana* had significant effect on conidial viability (Fig. 4). Conidial viability was significantly decreased if the *B. bassiana* culture was incubated at 34 °C, either for 24-hour-suspension culture (P = 0.00) or 48-hour-suspension culture (P = 0.00). Therefore, incubation temperature at 34 °C for 7 days for *B. bassiana* culture was capable to decrease the fungal conidial germination, although some local isolates of BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B could produce high percentage of germination.

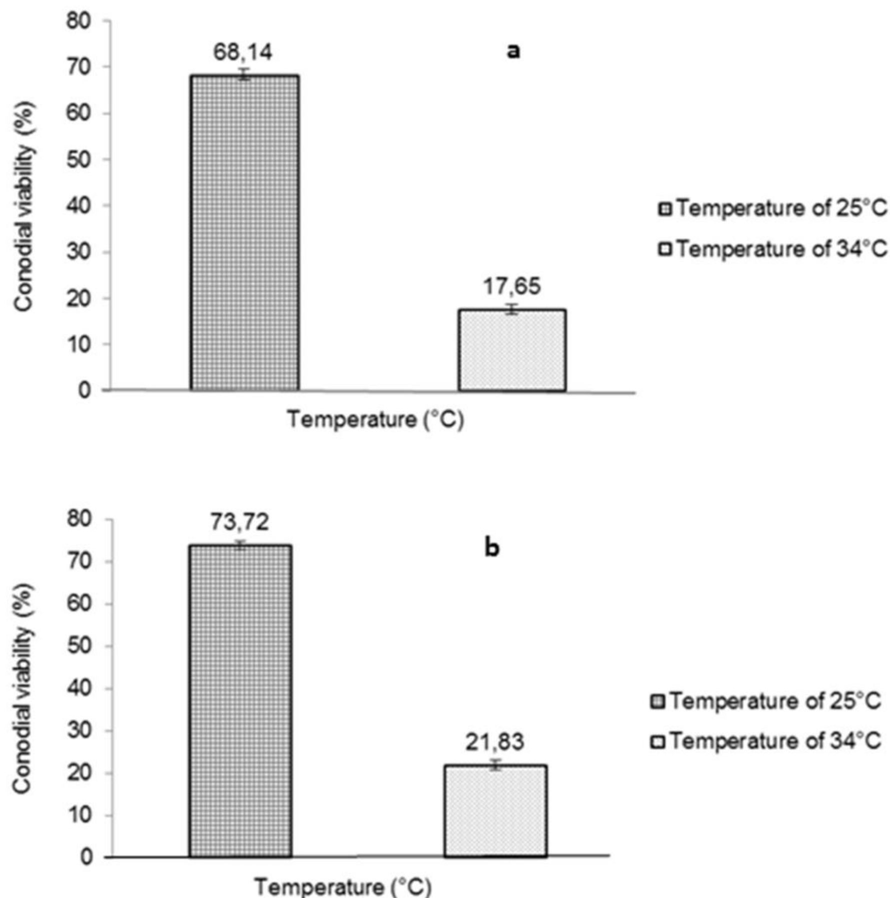

Fig. 4. Conidial viability of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C in 24-hour (P = 0.00) (a) and 48-hour suspension culture (P = 0.00) (b)

The shape of conidial germination of *B. bassiana* in this research was similar to description of conidial germination given by the following researcher. Conidial germination showed the following signs: broken conidial wall resulting in germ tube formation which had long stretch and increased in size or diameter of conidia (Oliveira, Pauli, Mascarin, & Delalibera, 2015; Safitri, Herlinda, & Setiawan, 2018). Percentage of conidial germination was used to measure conidial viability of the fungus. Conidial viability of *B. bassiana* at 25 °C was high on TS1D3A, BTmKt and BLePd isolates. However, the high conidial viability was found on TS1D3A, commercial BVR#, BTmPc, BBY, BTmPd, TSID3B and TS1D2B isolates at 34 ºC. The conidial viability of *B. bassiana* in 24-hour suspension or 48-hour suspension culture was decreased significantly at incubation temperature of 34 °C. The ideal temperature for the conidial germination of *B. bassiana* was in the range of 25 to 27 °C, but *B. bassiana* conidia could still germinate at 32 °C (Constanski et al., 2011). Salim, Md. Rawi, Ahmad, & Al-Shami (2015) had stated that the conidial germination of *B. bassiana* still occurred up to 33 °C. Local isolates of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B originated from soils and insects of South Sumatra and East Java, Indonesia still had high conidial viability at 34 °C. The conidial viability of these local isolates were equivalent to those of the commercial BVR# isolate. The conidial isolates that were capable to germinate at 34 °C were rarely occurred, but only high temperature resistant isolates that had the capability to germinate. According to Salim, Md. Rawi, Ahmad, & Al-Shami (2015) only superior strains of the fungi were able to germinate at above 33 ºC. The conidia of the local isolates in this experiment that were capable to germinate at 34 ºC had potential to be developed as active ingredients of bioinsecticides to control *N. lugens* in high temperature rice ecosystem, such as wetland or lowland rice ecosystems of South Sumatra, Indonesia. According to Siaga et al. (2019) the temperature occured in wetland or lowland rice ecosystems of South Sumatra was above 30 °C.

Virulence of *Beauveria bassiana*

The symptom of *N. lugens* nymphs infected by *B. bassiana* started to appear at the second day after being exposed to the fungal conidia with doses of 1 x 10^3 conidia per cm^2. The nymphs had slow movement

and finally stopped moving, whereas the healthy nymphs still actively moved on the rice stem. On the third day, some of the sick nymphs were dead and the other infected nymphs which were still alive became unable to move anymore with their legs and stylets attached on the rice stem. On the fourth and fifth days, the dead nymphs hung with their stylets still attached on the rice stem, while all their legs did not grip on the rice stem anymore. On the sixth day, the dead nymphs became hardened and stiff. On the seventh day, their bodies wrinkled, decayed with no smell and their integuments were coated with white color mycelia which gradually became brownish white to dark brown colors (Fig. 5).

Virulence of *B. bassiana* isolates against *N. lugens* nymphs was represented on the percentage of mortality and the lethal time to 50 % (LT_{50}) and 95 % (LT_{95}) mortality of *N. lugens* nymphs caused by *B. bassiana.* All isolates of *B. bassiana* tested in this experiment were pathogenic against the *N. lugens* nymphs. At temperature of 25 °C, mean mortality of *N. lugens* nymphs with magnitude of 96.67 % was found on TS1D2B isolate, whereas the lowest mean mortality with magnitude of 20 % was found on BPcPd2 isolate (Table 4). The highest value of mean mortality of *N. lugens* nymphs at 34 °C was found on TS1D2B isolate (43.33 %), wehereas the lowest value of mean mortality of *N. lugens* nymphs was found on Bws Pantura isolate (5 %). However, mortality of *N. lugens* nymphs caused by *B. bassiana* was not significantly different among all isolates either at temperarture of 25 or 34 °C. The percentage of *N. lugens* mortality was significantly decreased when fungal incubation temperature was increased from 25 to 34 °C (P = 0.03) (Fig. 6). Therefore, the incubation temperature at 34 °C for 7 days was significantly decreased the virulence of some *B. basiana* isolates.

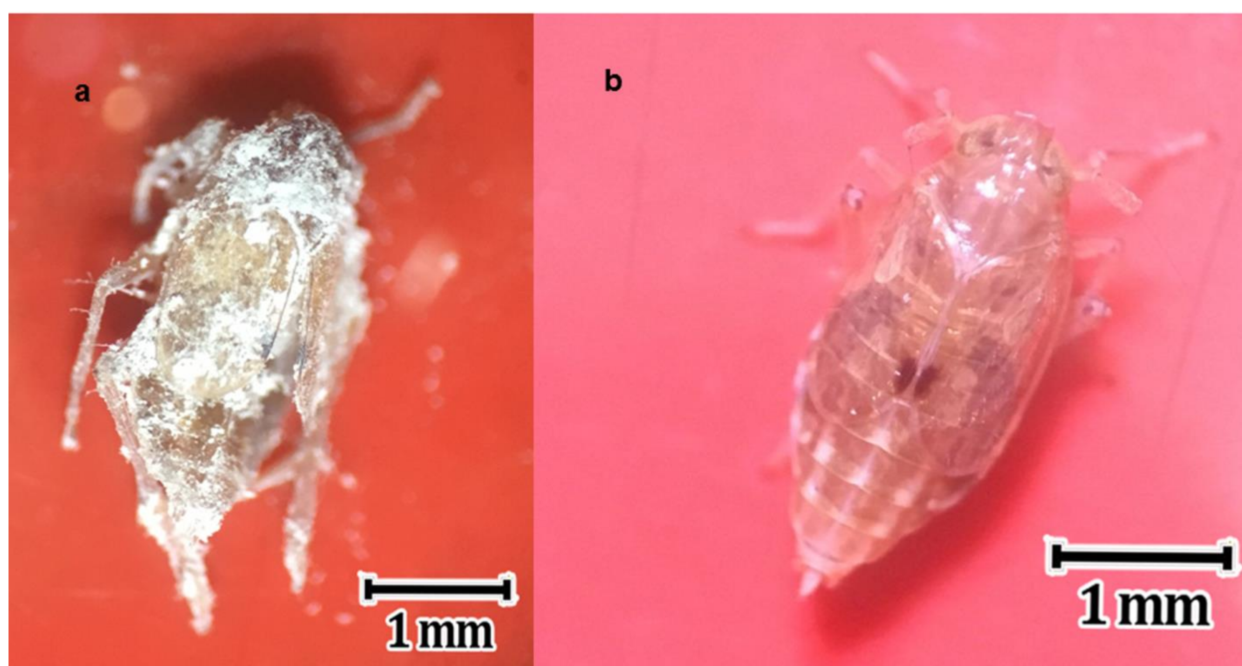

Fig. 5. *Nilaparvata lugens* infected by *Beauveria bassiana* (a) and the healthy one (b)

Table 4. Virulence of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C against *Nilaparvata lugens* nymphs

Isolate codes	Mortality of *Nilaparvata lugens* nymphs ± SD (%)	
	25 °C	34 °C
BPcMs	65.00±5.00	21.67±5.77
BTmKt	26.67±10.41	13.33±14.43
BTmPc	43.33±7.64	28.33±7.64
Bws Pantura	30.00±18.03	5.00±5.00
BBY	38.33±15.28	16.67±7.64
BTmPe	28.33±10.41	21.67±17.56
BTmMa	28.33±14.43	25.00±18.03
BTmSo	56.67±10.41	40.00±18.03
BTmSr	35.00±5.00	35.00±5.00
BuBj	45.00±21.79	26.67±28.87
725HaJ	23.33±2.89	20.00±5.00
715HhB	15.00±5.00	6.67±7.64
BTmPd	26.67±7.64	20.00±15.00
BTmTs	26.67±10.41	13.33±2.89

BTmkbc	28.33±15.28	28.33±15.28
BPcPd2	20.00±10.00	10.00±10.00
BPcPd	25.00±10.00	25.00±10.00
BMkMs	26.67±14.43	26.67±14.43
BTmTr	31.67±24.66	20.00±5.00
Natural BVR[#]	43.33±12.58	36.67±7.64
BTmGa	30.00±5.00	30.00±5.00
BLePd	26.67±11.55	23.33±5.77
TS1D3A	90.00±13.23	38.33±11.55
TSID3B	58.33±7.64	43.33±14.43
TS1D2A	81.67±20.21	41.67±15.28
TS1D2B	96.67±5.77	43.33±18.93
ANOVA F-value	0.29ns	0.16ns
P value (0.05)	0.99	1.00

Remarks: ns = not significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test.

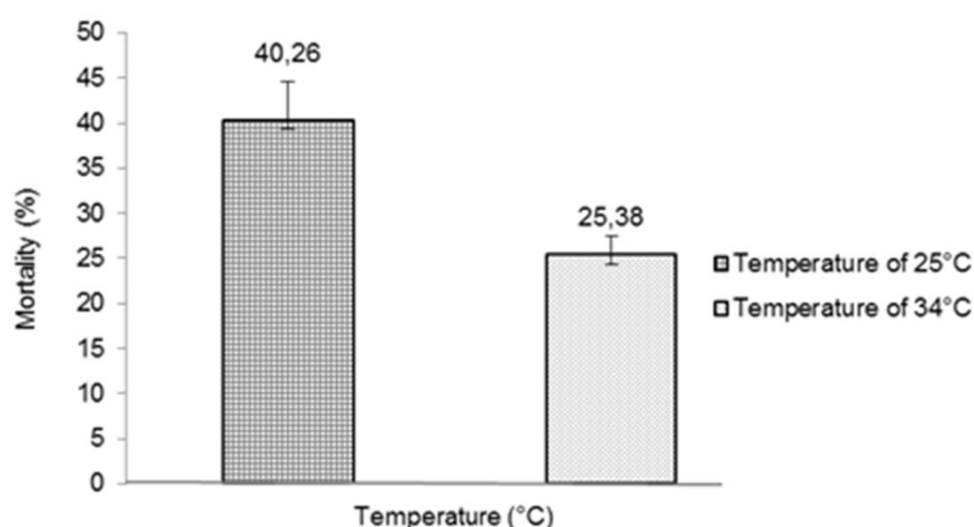

Fig. 6. Mortality of *Nilaparvata lugens* caused by *Beauveria bassiana* culture incubated at 25 and 34 °C (P = 0.003)

Table 5. LT_{50} and LT_{95} of *Nilaparvata lugens* nymphs caused by *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	LT_{50} (days) (95 % fiducial limits)		LT_{95} (days) (95 % fiducial limits)	
	25 °C	34 °C	25 °C	34 °C
BPcMs	2.62 (1.68 – 3.72)	4.95 (3.71 – 6.14)	7.43 (5.66 – 9.15)	11.40 (9.43 – 12.78)
BTmKt	4.18 (3.37 – 4.82)	7.48 (5.77 – 8.76)	9.00 (8.35 – 10.25)	13.93 (11.63 – 16.56)
BTmPc	3.86 (2.58 – 5.28)	4.87 (4.26 – 5.20)	8.58 (7.71 – 10.03)	11.33 (9.97 – 13.00)
Bws Pantura	4.31 (2.86 – 6.85)	10.40 (7.22 – 14.22)	9.13 (8.26 – 10.83)	16.85 (12.94 – 22.01)
BBY	2.93 (2.60 – 3.33)	5.96 (3.62 – 8.12)	7.75 (6.84 – 8.76)	12.41 (9.34 – 15.91)
BTmPe	3.88 (3.65 – 4.23)	5.92 (3.11 – 10.56)	8.69 (8.21 – 9.08)	12.37 (8.96 – 18.36)
BTmMa	2.76 (2.01 – 4.16)	2.99 (1.36 – 4.42)	7.57 (6.09 – 9.19)	9.45 (7.08 – 12.22)
BTmSo	3.31 (2.68 – 4.42)	3.09 (2.56 – 3.68)	8.13 (7.71 – 8.40)	9.55 (8.27 -11.48)
BTmSr	2.57 (1.94 – 2.95)	2.92 (2.40 – 3.60)	7.39 (6.94 – 8.26)	9.38 (8.11 – 10.56)
BuBj	2.68 (1.76 – 4.16)	6.43 (1.45 – 10.35)	7.49 (6.09 – 9.19)	12.89 (7.17 – 18.14)
725HaJ	3.19 (2.73 – 3.76)	5.65 (4.35 – 7.04)	8.00 (7.05 – 8.79)	12.00 (11.41 – 12.45)
715HhB	5.06 (2.98 – 6.76)	9.00 (6.92 – 10.47)	9.88 (6.96 – 12.19)	15.46 (12.77 – 17.42)
BTmPd	2.24 (1.59 – 3.24)	5.02 (2.73 – 8.85)	7.06 (5.88 – 8.67)	11.47 (8.44 – 16.65)
BTmTs	3.93 (3.07 – 4.98)	6.55 (5.44 – 8.12)	8.75 (7.05 – 10.40)	13.01 (11.15 – 15.91)
BTmkbc	4.61 (3.24 – 5.32)	3.31 (1.21 – 6.13)	9.13 (7.77 – 10.31)	9.76 (6.92 – 13.93)
BPcPd2	4.21 (2.73 – 6.82)	8.15 (2.75 – 14.22)	9.02 (7.05 – 11.85)	14.61 (8.46 – 22.01)
BPcPd	4.39 (3.09 – 5.65)	4.37 (3.09 – 6.51)	9.20 (8.40 – 10.68)	10.91 (8.80 – 12.64)
BMkMs	4.19 (2.66 – 7.09)	3.95 (2.23 – 6.65)	9.01 (6.64 – 12.12)	10.74 (7.95 – 12.51)
BTmTr	3.82 (2.91 – 4.65)	4.66 (3.97 – 5.09)	8.64 (6.89 – 10.08)	11.12 (9.82 – 12.72)
Natural BVR[#]	4.12 (3.37 – 5.44)	4.06 (3.65 – 4.65)	8.94 (7.35 – 10.47)	10.51 (9.71 – 11.45)
BTmGa	3.68 (2.93 – 4.36)	3.96 (2.94 – 4.66)	8.50 (8.35 – 8.79)	10.41 (8.66 – 12.08)
BLePd	3.90 (3.33 – 4.59)	3.79 (3.50 – 3.97)	8.72 (8.36 – 9.22)	10.25 (9.62 – 11.29)
TS1D3A	2.99 (2.46 – 3.55)	4.17 (4.07 – 4.32)	7.80 (6.94 – 8.98)	10.63 (9.79 – 11.92)
TSID3B	2.34 (1.63 – 3.72)	3.92 (3.23 – 4.91)	7.16 (5.61 – 9.15)	10.38 (9.48 – 11.02)
TS1D2A	2.41 (1.19 – 3.10)	5.80 (4.17 – 7.26)	7.23 (6.62 – 7.97)	12.26 (9.88 – 13.78)

TS1D2B	4.19 (3.05 – 5.28)	4.35 (3.48 – 4.82)	9.01 (8.23 – 10.31)	10.67 (10.13 -11.28)
ANOVA F-value	0.84ns	2.08ns	0.84ns	1.21ns
P value (0.05)	0.68	0.01	0.68	0.28

Remarks: ns = not significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test.

All isolates of *B. bassiana* were pathogenic against *N. lugens* nymphs. The mortality of the *N. lugens* nymphs caused by all isolates was high. However, virulence of *B. bassiana* isolates against *N. lugens* nymphs was significantly decreased if *B. bassiana* culture was incubated at 34 °C. Virulence of *B. bassiana* was decreased at 34 °C due to the decrease of conidial viability. Virulence of *B. bassiana* was affected by the conidial viability (Ghany, 2015). The higher of the capability of conidia germinate, the higher the probability of germ tube formation of the conidia penetrate insect cuticle (Butt, Ibrahim, Ball, & Clark, 1994; Fernandes, Rangel, Moraes, Bittencourt, & Roberts, 2007). The local isolates from South Sumatra of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B in this study still had high conidial viability at 34 °C which in turn cause the high percentage of *N. lugens* mortality. These local isolates could adapt to high temperature of 34 °C and could be chosen as candidates for biocontrol agents of *N. lugens* in wetland or lowland rice ecosystems in Indonesia.

At fungal incubation temperature of 25 °C, mean of LT_{50} values ranged from 2.24 to 5.06 days and the shortest time was found on BTmPd isolate, whereas the longest time was found on 715 HHBanyuwangi isolate (Table 5). Mean of LT_{50} values caused by *B. bassiana* incubated at 34 °C ranged from 2.92 to 10.40 days and the shortest time was found on isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. They were isolates from South Sumatra. Whereas, the longest time was found on Bws Pantura isolate. TSID3B isolate culture incubated at 25 ºC had the shortest lethal time values of 2.34 days (LT_{50}) and 7.16 days (LT_{95}). However, LT_{50} values were not significantly different among all isolates of *B. bassiana* and similar trend was also occurred on LT_{95}. Incubation temperature for *B. bassiana* culture affects LT_{50} or LT_{95} values. Mean of LT_{50} or LT_{95} values was significantly longer on *B. bassiana* incubated at 34 ºC than that of *B. bassiana* incubated at 25ºC (Fig. 7). Therefore, incubation temperature at 34 ºC for 7 days for *B. bassiana* culture could extend the lethal time to 50 % and 95 % mortality of *N. lugens* nymphs.

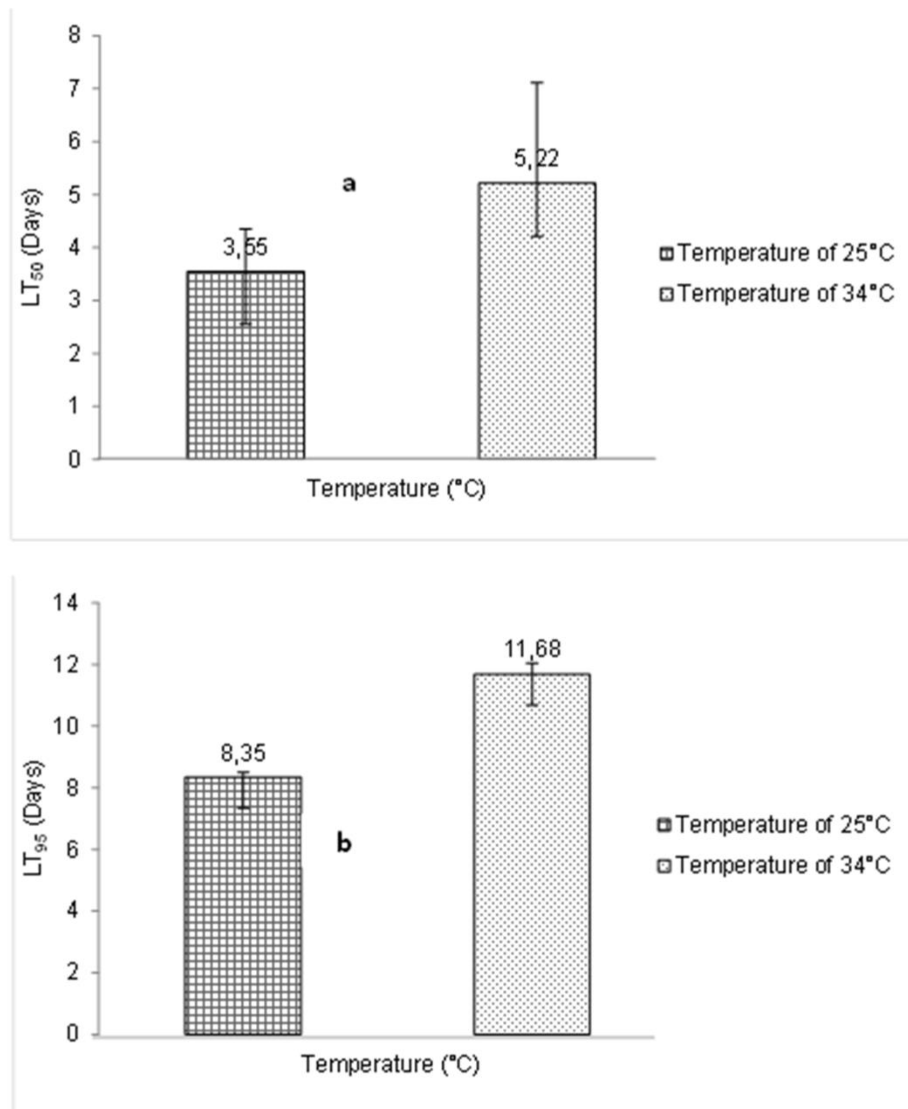

Fig. 7. LT50 (P = 0.00) (a) and LT95 (P = 0.00) (b) caused by *Beauveria bassiana* culture incubated at 25 and 34 °C against Nilaparvata lugens

The incubation temperature at 34 °C for *B. bassiana* culture could prolong the LT_{50} and LT_{95} of *N. lugens* nymphs, but some isolates that still have short LT_{50} or LT_{95} value were consisted of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. LT_{50} or LT_{95} value of *N. lugens* caused by *B. bassiana* was affected by conidial viability of the fungus. The lower percentage of conidial germination caused longer time for the fungus to invade the whole body of insect hosts (Fernandes, Rangel, Moraes, Bittencourt, & Roberts, 2007). In this research, the isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B at 34ºC causing the lethal time to 50% mortality of *N. lugens* nymphs were 3−4 days. The normal time required by conidia to kill insect host was 4−10 days (Ghany, 2015). The time required by *B. bassiana* to kill *N. lugens* nymphs in this study were shorter because the insect hosts were more sensitive than other insect host species. Conidia requires certain time to germinate on insect cuticle surface and subsequently mycelia penetrates into body cavity (Fernandes, Rangel, Moraes, Bittencourt, & Roberts, 2007). Then, the host insect will die whitin 4 days (Butt, Ibrahim, Ball, & Clark, 1994). Next, the fungus yields thousands of new spores on the dead body (Ghany, 2015).

CONCLUSION

It can be concluded from this study that at germination temperature of 34 °C, some isolates of *B. bassiana* originate from soils or insects, especially from South Sumatra, could produce high conidial density and viability as well as high virulent against *N. lugens* nymphs. The importance of this finding showed that some isolates were still virulent although their culture were incubated at 34 °C for 7 days. Therefore, the isolates can be used as promising candidates for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as tidal lowland and lowland swamp ecosystems in Indonesia.

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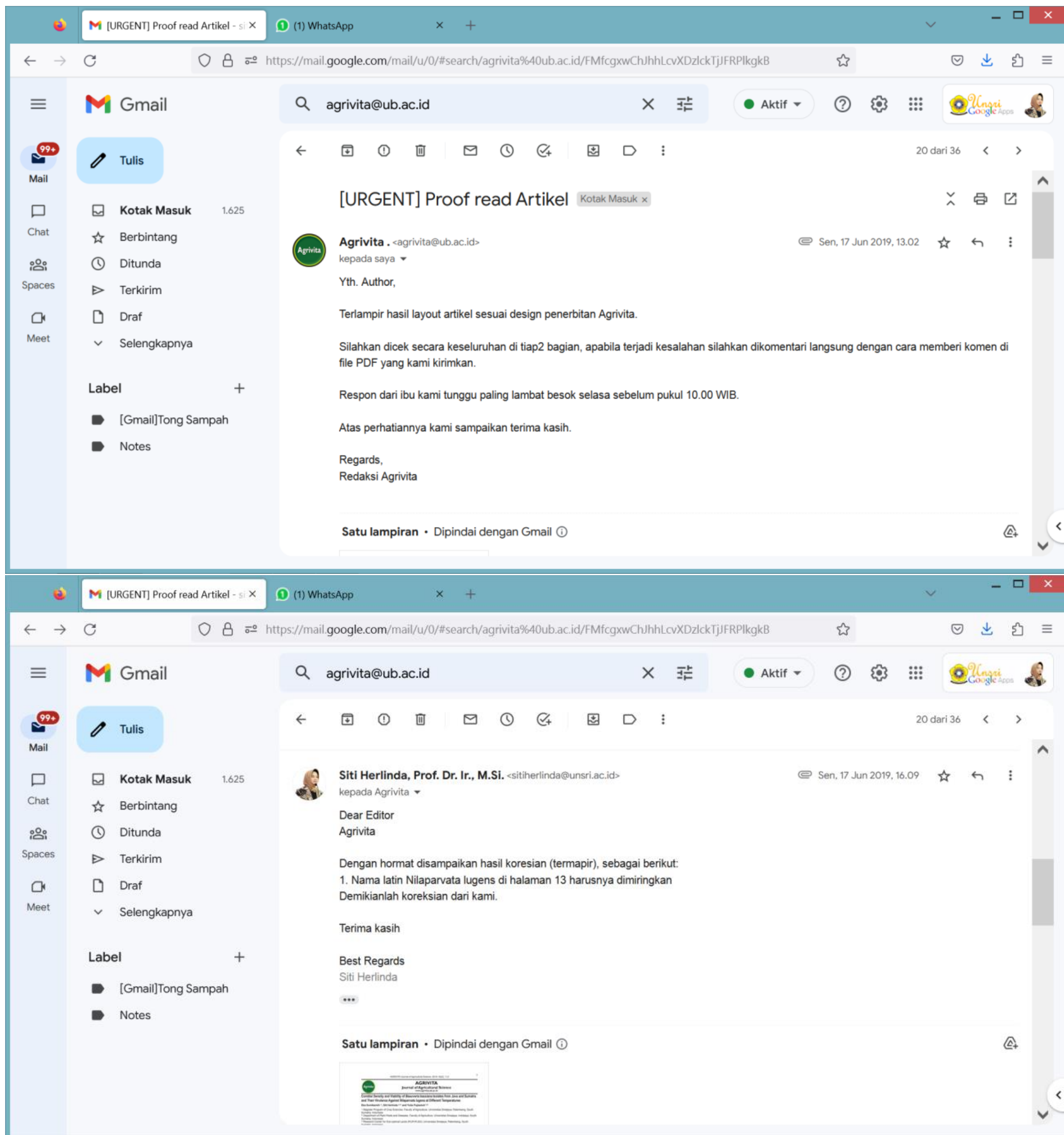

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Conidial Density and Viability of *Beauveria bassiana* Isolates from Java and Sumatra and Their Virulence Against *Nilaparvata lugens* at Different Temperatures

Eka Sumikarsih [1)], Siti Herlinda [2,3*)] and Yulia Pujiastuti [2,3)]

[1)] Magister Program of Crop Sciences, Faculty of Agriculture, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia
[2)] Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, South Sumatra, Indonesia
[3)] Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang, South Sumatra, Indonesia

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*) Corresponding author:
E-mail: sitiherlinda@unsri.ac.id

ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* can cause direct damage and transmit rice diseases. *Beauveria bassiana* is used to control BPH, however the success of the fungal efficacy on rice fields is affected by external factors, such as temperature. This research aimed to evaluate the conidial viability and density of *B. bassiana* isolates from Java and Sumatra, exposed to 25 and 34°C and their virulence against BPH nymphs. Twenty six isolates of *B. bassiana* cultures incubated at 25 and 34°C for 7 days were observed on their conidial density, viability, and virulence against BPH nymphs. The incubation temperature of 34°C was able to decrease conidial density and viability, and virulence of the isolates. However, some isolates of *B. bassiana* originated from soils or insects in Sumatra, especially from South Sumatra still produced high conidial density and viability as well as high virulent against BPH nymphs, such as TS1D3A, TSID3B, TS1D2A and TS1D2B isolates. The TS1D2B isolate incubated at 34°C still caused the highest percentage of BPH mortality (43.33%) among other isolates. Therefore, the isolates can be used as promosing candidate for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as wetland or lowland rice ecosystems in Indonesia.

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae) is the most serious insect pest which sucks phloem sap on stems of rice (*Oryza sativa* L.) (Daravath & Chander, 2017; Herlinda et al., 2018). A direct damage caused by this BPH results in hampered growth of rice producing 'hopperburn' (Dharshini & Siddegowda, 2015). In addition, BPH can also as an insect vector that transmits rice diseases, such as the ragged stunt and grassy stunt virus (Dietzgen, Mann, & Johnson, 2016; Zheng, Mao, Xie, & Wei, 2014). The attack of BPH do not only occure in Indonesia, but also attack rice in several Asian countries, such as China, Vietnam, Thailand, India, Pakistan, Malaysia and Philippine (Catiding et al., 2009; Hu et al., 2014). BPH also attacked rice in Texas (Leavengood, Bartlett, & Vitanza-Hedman, 2017).

The effort to control population of *N. lugens* had been done through synthetic chemical control (Baehaki & Suparno, 2018; Liu et al., 2013; Zhang et al., 2014) and biological control by using entomopathogenic fungi, such as *Beauveria bassiana* (Lee et al., 2015; Li, Lin, Li, Xu, & Feng, 2012) and *Metarhizium anisopliae* (Chinniah, Ravikumar, Kalyanasundaram, & Parthiban, 2016) have been proven to be effective agents to control the BPH. *B. bassiana* could kill BPH more than 80% (Lee et al., 2015; Li et al., 2014) and to kill the eggs

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of BPH as well as to disturb adult stage proliferation (Li, Lin, Li, Xu, & Feng, 2012). *B. bassiana* is also not harmful toward natural enemies of the BPH (Firouzbakht, Zibaee, Hoda, & Sohani, 2015; Gholamzadeh-Chitgar, Hajizadeh, Ghadamyari, Karimi-Malati, & Hoda, 2017).

Although *B. bassiana* had proven to be effective in controlling the BPH, the success of its efficacy on rice fields was affected by many external factors, such as temperature (Ghany, 2015). Extremely high temperature can results in death of the fungus (Ottati-de-Lima et al., 2014). The optimum temperature for growth of the entomopathogenic fungi usually is in the range of 25 to 30 ºC (Bugeme, Maniania, Knapp, & Boga, 2008). Most of entomopathogenic fungi tolerate to temperatures in the range of 0 to 40 °C (Ghany, 2015), but certain strains of entomopathogenic fungi can only survive at temperatures below 35 °C (Constanski et al., 2011). The production of colony and conidial density of *B. bassiana* is significantly decreased if the temperature during fungal incubation increases from 30 to 35 °C (Ottati-de-Lima et al., 2014) and all isolates are dead at temperature of 36 °C (Ottati-de-Lima et al., 2014; Pham, Kim, Kim, & Kim, 2009). Fungal germination is also decrease at temperature above 30 °C (Pham, Kim, Kim, & Kim, 2009) with the highest level up to 33 °C (Salim, Md. Rawi, Ahmad, & Al-Shami, 2015), whereas temperature of 25 °C is an ideal temperature for the fungal germination (Lohse, Jakobs-Schönwandt, & Patel, 2014). Virulence of the entomopathogenic fungi can be affected by temperature (Bugeme, Maniania, Knapp, & Boga, 2008; Constanski et al., 2011; Ghany, 2015; Ottati-de-Lima et al., 2014; Satpathi, Acharjee, & Saha, 2016; Tefera & Pringle, 2003). Each strain/isolate or species of the entomopathogenic fungi also has different optimum temperature and tolerance level to temperature. Entomopathogenic fungal strains that can survive at extremely high temperature of above 33 °C are superior strains (Salim, Md. Rawi, Ahmad, & Al-Shami, 2015). These superior strains can be used as candidates to control the BPH in tropical ecosystems, such as agroecosystems in Indonesia. Therefore, the objectives of this research were to evaluate the conidial viability and density of *B. bassiana* isolates form Java and Sumatra exposed to 25 and 34 °C temperatures and their virulence against *N. lugens* nymphs.

MATERIALS AND METHODS

Study Site

This research was conducted at Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, from September 2017 to April 2018. Isolates used in this research were isolates that collected from soil of lowland swamps, tidal lowlands, peatlands, and highlands in South Sumatra, whereas isolates from soil and infected insects obtaining from other provinces used as comparison as well as one commercial isolate as control (Table 1). The species of the fungus was identified by Dr. Suwandi, a microbiologist from Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya. The average room temperature during the experiment was 30.5 °C and the average relative humidity was 84.30% within the laboratory during the experiment.

Preparation of Test Insects

Adults and nymphs of N. lugens were collected from fields of rice at Indralaya, South Sumatra from September 2017 to March 2018 and brought to the Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya for identification. Then, the nymph and adults were reared and maintained on 5 clumps of 10-day-old rice grown within bucket (bottom diameter of 20 cm, upper diameter of 25 cm, and height of 20 cm) within a greenhouse at temperature range of 30 to 35 °C. The rice was put in wire mesh enclosure (height of 100 cm, length of 50 cm, and width of 50 cm). Within one of wire mesh enclosure, 10 pairs of the adults were released in order to infect the rice for 10 days and subsequently the infected rice crops were substituted with the healthy one and this propagation was done on 30 cages. Fresh and healthy rice were given to newly emerged nymphs of N. lugens and this was maintained up to at least five generations. The sixth generation and henceforth generations were used and selected for the fourth instar for bioassay in this experiment.

Isolates Preparation of *Beauveria bassiana*

All isolates used in this study were previously fitted by using living insects of *Tenebrio molitor*. Fresh cultures of *B. bassiana* were started by inoculating Sabouraud Dextrose Agar (SDA) (Oxoid) with the third larvae of *T. molitor*. Prior to inoculation, *B.*

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bassiana and *T. molitor* were previously sterilized by using modified method from Nuraini, Setyaningsih, & Susilowati (2017). Subsequently, the fungal culture was incubated for 7 x 24 hours in order to produce sufficients numbers of fungal colony. The *B. bassiana* culture that had previously fitted was then used for subsequent observation. The fresh *B. bassiana* culture was cut with dimension of 10 mm x 10 mm for recultured into SDA medium which would be used for subsequent observation consisting of conidial density and viability as well as virulence test.

Observation of Conidial Density and Viability

Twenty six isolates of the *B. bassiana* culture were grown on SDA medium and then each isolate was incubated at constant temperature of 25 and 34 °C within incubator for 7 x 24 hours using three replications. The ideal temperature of 25 °C and extremely high temperature of 34 °C in this research were chosen for culturing of *B. bassiana* for 7 days. Both temperature were chosen because the temperature of 25 °C is ideal temperature for culture *B. bassiana* (Bugeme, Maniania, Knapp, & Boga, 2008), whereas temperature higher than 33 °C is extremely high temperature (Salim, Md. Rawi, Ahmad, & Al-Shami, 2015). Conidia of all isolates at the 8th day were counted in term of their density of the 7 day *B. bassiana* culture. Calculation of conidial density was started with fungal suspension production by harvesting 10 mm x 10 mm (1 cm^2) 7-day *B. bassiana* culture which followed by 10 ml addition of sterile distilled water. The suspension was vortexed using turbo mixer for 20 seconds in order to produce homogenous conidial suspension. This suspension culture was diluted through addition of 9 ml sterile distilled water into 1 ml *B. bassiana* suspension culture, homogenized. Subsequently, the last suspension culture was counted in term of its conidial density under a compound microscope at 400x magnification that had been equipped with haemocytometer. This treatments were arranged in completely randomized design and replicated three times.

Table 1. *Beauveria bassiana* isolates used in this research

Isolate codes	Species of fungi	Source (host insects or soil)	Origin (village or city), Province in Indonesia
BPcMs	*Beauveria bassiana*	*Pseudoplusia chalcites*	Muarasiban, South Sumatra
BTmKt	*Beauveria bassiana*	Fresh swamp soils	Kenten, South Sumatra
BTmPc	*Beauveria bassiana*	Fresh swamp soils	Indralaya, South Sumatra
Bws Pantura	*Beauveria bassiana*	*Leptocorisa acuta*	Pantura, West Java
BBY	*Beauveria bassiana*	*Hypothenemus hampei*	Jember, East Java
BTmPe	*Beauveria bassiana*	Fresh swamp soils	Pemulutan, South Sumatra
BTmMa	*Beauveria bassiana*	Fresh swamp soils	Mariana, South Sumatra
BTmSo	*Beauveria bassiana*	Fresh swamp soils	Soak, South Sumatra
BTmSr	*Beauveria bassiana*	Tidal lowland soils	Srikaton, South Sumatra
BuBj	*Beauveria bassiana*	*Alphitobius diaperinu*	Jarai, South Sumatra
725HaJ	*Beauveria bassiana*	*Helopeltis antonii*	Jember, East Java
715HhB	*Beauveria bassiana*	*Hypothenemus hampei*	Banyuwangi, East Java
BTmPd	*Beauveria bassiana*	Highland soils	Pagardin, South Sumatra
BTmTs	*Beauveria bassiana*	Highland soils	Mulia Sari, South Sumatra
BTmkbc	*Beauveria bassiana*	*Chrysodeixis chalcites*	Curup, Bengkulu
BPcPd2	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BPcPd	*Beauveria bassiana*	*Chrysodeixis chalcites*	Pagardin, South Sumatra
BMkMs	*Beauveria bassiana*	Highland soils	Muarasiban, South Sumatra
BTmTr	*Beauveria bassiana*	Tidal lowland soils	Telang Rejo, South Sumatra
Natural BVR#	*Beauveria bassiana*	-	-
BTmGa	*Beauveria bassiana*	Fresh swamp soils	Gandus, South Sumatra
BLePd	*Beauveria bassiana*	*Lipaphis erysimi*	Pagardin, South Sumatra
TS1D3A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TSID3B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2A	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra
TS1D2B	*Beauveria bassiana*	Peat soils	Talang Dabok,South Sumatra

Remarks: #Control = commercial products, - unkown source

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Table 2. Conidial density of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial density ± SD (10^7 conidia per cm^2)	
	25 °C	34 °C
BPcMs	27.34±2.34 m	2.71±0.72 bcd
BTmKt	9.81±0.09 bcde	1.19±0.05 abcd
BTmPc	13.01±0.77 efgh	1.28±0.21 abcd
Bws Pantura	16.87±0.72 hijk	1.61± 0.91 abcd
BBY	19.25±2.49 jkl	1.27±0.24 abcd
BTmPe	12.26±0.48 defg	1.04±0.19 ab
BTmMa	10.93±0.79 cdef	1.15±0.26 abcd
BTmSo	28.21±1.63 m	2.30±0.75 abcd
BTmSr	6.76±0.39 a	1.50±0.66 abcd
BuBj	11.42±0.36 cdef	1.83±0.49 abcd
725HaJ	16.26±3.76 ghij	1.09±0.14 abc
715HhB	7.73±1.43 ab	1.11±0.15 abc
BTmPd	11.35± 0.15 cdef	1.43±0.02 abcd
BTmTs	12.49±1.49 efgh	1.45±0.35 abcd
BTmkbc	14.17±2.44 fghi	1.33±0.39 abcd
BPcPd2	9.40±1.19 bcd	0.97±0.19 a
BPcPd	9.87±1.06 bcde	1.73±0.98 abcd
BMkMs	14.45±0.91 fghi	1.20±0.20 abcd
BTmTr	11.79±1.91 efg	1.24±0.36 abcd
Natural BVR#	11.72±0.28 efg	1.55±0.55 abcd
BTmGa	13.07±0.99 efgh	1.54±0.67 abcd
BLePd	8.44±0.25 abc	1.45±0.31 abcd
TS1D3A	21.14±0.58 jklm	3.03±0.79 d
TSID3B	22.82±0.13 klm	2.80±0.24 cd
TS1D2A	28.46±2.64 m	3.00±1.35 cd
TS1D2B	24.02±0.78 lm	3.05±0.87 d
ANOVA F-value	49.05*	3.98*
P value (0.05)	1.7×10^{-37}	1.61×10^{-6}
Tukey's HSD test	0.1383	0.4184

Remarks: * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at $P < 0.05$ according to HSD test

The calculation of conidial viability was based on percentage of conidial germination. The conidial germination was observed from the 7-day *B. bassiana* culture which incubated at 25 or 34 °C and grown on one layer of SDA medium according to the method of Bugeme, Maniania, Knapp, & Boga (2008). The 7-day *B. bassiana* suspension culture was scratched with magnitude of 0.1 ml on SDA plates. A sterile microscope cover slip was placed on each plate, then each suspension culture was incubated for 24 and 48 hours at temperature of 25 °C. Furthermore, the germinated conidia was observed under microscope and calculation was done in term of numbers of germinated conidia. The germination percentage was determined from 100-spores for each plate using the compound microscope. These treatments were arranged in completely randomized design and replicated three times.

Bioassay Procedure

Bioassays by modification of Trizelia & Nurdin (2010) method were conducted to determine the virulence of *B. bassiana* isolates againts *N. lugens* nymphs. The 26 isolates of *B. bassiana* were incubated at 25 and 34 °C for 7 days and their conidia were harvested. Each isolates of *B. bassiana* was topically sprayed with 10 ml of a concentration

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of 1 x 10^3 conidia per cm^2 on 25 fourth-nymphs of *N. lugens* placed on a filter paper in petri dishes. Then, the nymphs of *N. lugens* were placed into 20 healthy two-weeks rice stems. This treatments were arranged in completely randomized design and replicated three times. Numbers of dead *N. lugens* nymphs was recorded every 12 hours for 11 days period which used to determine the percentage of mortality and lethal time to 50% (LT_{50}) and 95% (LT_{95}) mortality of *N. lugens* nymphs. The behaviour change of *N. lugens* infested by *B. bassiana* was observed and recorded daily until the insects were dead and mycelia cover all of their bodies. The dead nymphs were transferred into petri dishes lined with moist-sterile filter paper to allow the growth of the *B. bassiana* on the surface of the cadaver.

Data Analysis

Data of conidial density and viability, and percentage of mortality among treatments were analyzed by using analysis of variance (ANOVA). If there were differences among the data of each isolate, then Honestly Significant Different (HSD) test at 5% was conducted by using program software of SAS University Edition 2.7 9.4 M5. The data between temperature were compared using t test. LT_{50} and LT_{95} values were calculated using probit analysis.

RESULTS AND DISCUSSION

Conidial Density and Viability of Beauveria bassiana

B. bassiana culture that was incubated for 7 days at temperature of 25 °C showed the highest conidial density on BTmSo isolate and was not significantly different from that of BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B isolates (Table 2). At incubation temperature of 34 °C, the highest value of conidial density was found on TS1D2B isolate and was significantly different than that of BPcPd2 isolate which had the lowest conidial density value. Conidial density of all isolates at 25 °C were siginificantly higher than the isolates at 34 °C (P = 0.00) (Fig. 1). Conidial density significantly decreased with the increase of the fungal incubation temperature and followed by more hampered of fungal colony growth. Fungal culture incubated for 7 days at 25 °C had normal growth with colony diameter in the range of 50 to 90 mm, whereas colony of *B. bassiana* incubated at 34 °C only had diameter in the range of 15 to 30 mm (Fig. 2). Incubation temperature of 34 °C for *B. bassiana* culture was able to decrease conidial density and colony growth of the fungus; however some isolates (TS1D3A, TSID3B, TS1D2A and TS1D2B isolates) still achieved high conidial density and colony growth.

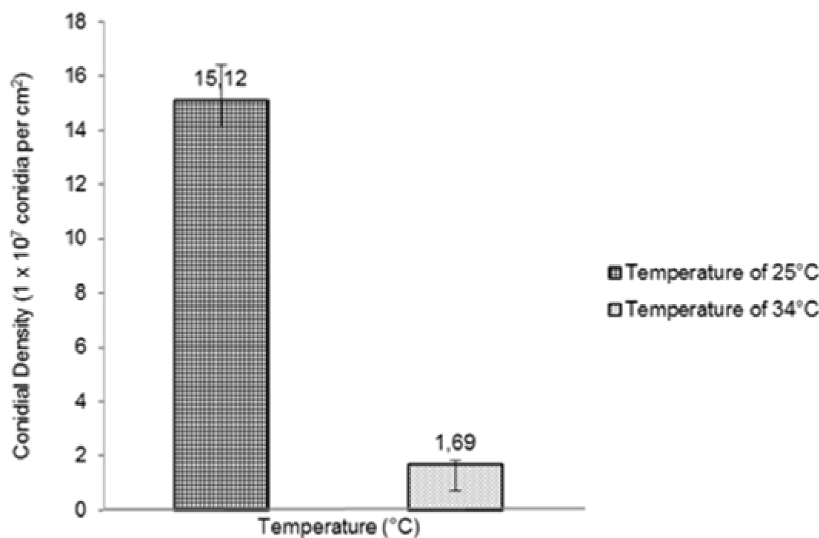

Fig. 1. Conidial density of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C (P = 0.00)

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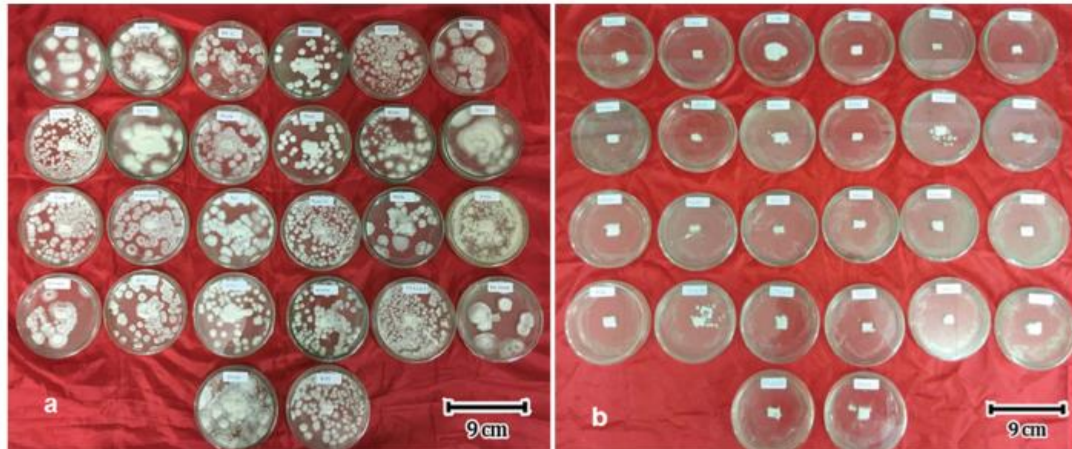

Fig. 2. Colony growth of *Beauveria bassiana* culture incubated for 7 days at 24 °C (a) and 34 °C (b) (90 mm diameter of petri dish).

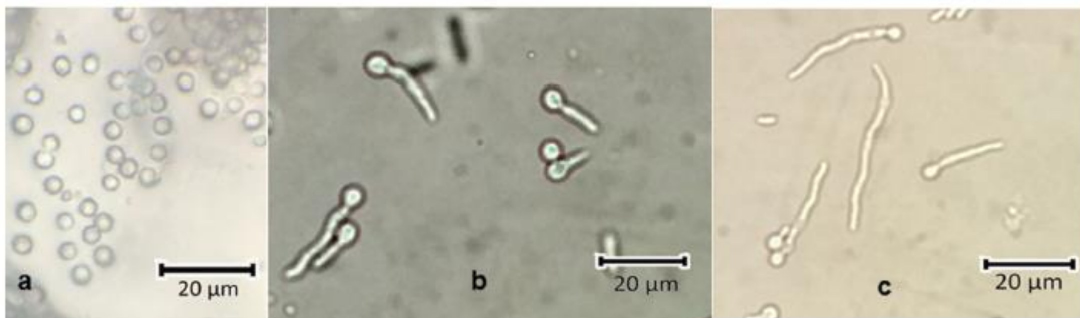

Fig. 3. Conidia of *Beauveria bassiana* (a), viable conidia at 24-hour (b) and 48-hours (c) suspension culture of *Beauveria bassiana* (400x magnification)

Conidial density values of *B. bassiana* isolate cultures incubated at 25 °C were all high, but higher values were found on isolates of BtmSo, BPcMs, TS1D3A, TSID3B, TS1D2A and TS1D2B. They were isolates from South Sumatra. Conidial density of all isolates were siginificantly decrease if the isolates were incubated at 34 °C. However, only TS1D2B isolate still had high conidial density at incubation temperature of 34 °C. The conidial density decreased at 34 °C due to the lower production of conidia per cm^2 in agar medium which indicated by hampered colony growth of *B. bassiana* (Fig. 2). The diameter of colony growth reached 90 mm at 25 °C, whereas the diameter of colony growth at 34 °C was only 15-30 mm. Ottati-de-Lima et al. (2014) had stated that *M. anisopliae* could yield optimal colonies in liquid medium from 25 to 30 °C and temperature of 35 °C was detrimental to colony growth of the fungus. High spore or conidial production of *B. bassiana* occurred at 25-27 °C (Pham, Kim, Kim, & Kim, 2009). The incubation temperature of 34 °C in this research could decrease the conidial density and colony growth of *B. bassiana*.

Viable or germinate conidia was characterized by the following aspects: the change of size and form of conidia compared to size and form of normal conidia (Fig. 3a), conidial wall broke and produced germ tube and followed by elongation of the germ tube (Fig. 3b and 3c). Percentage of conidial germination was used to determine conidial viability. At incubation temperature of 25 °C, the highest value of conidial viability of 24-hour-suspension culture was found on TS1D3A isolate and was not significantly different from other isolates, except BTmKt and BLePd isolates (Table 3). However, the highest value of conidial viability at temperature of

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34 °C was found on Natural BVR# isolate in control of commercial product and was not significantly different from other five isolates consisting of BTmPc, BBY, BtmPe, BTmPd and BTmkbc. Conidial viability of 48-hour-suspension culture at 25 °C for all isolates were high and not significantly different among isolates. Nevertheless, isolates that had the highest conidial viability at 34 °C was Natural BVR# isolate and was not significantly different from BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B isolates. The temperature increase during incubation of *B. bassiana* had significant effect on conidial viability (Fig. 4). Conidial viability was significantly decreased if the *B. bassiana* culture was incubated at 34 °C, either for 24-hour-suspension culture (P = 0.00) or 48-hour-suspension culture (P = 0.00). Therefore, incubation temperature at 34 °C for 7 days for *B. bassiana* culture was capable to decrease the fungal conidial germination, although some local isolates of BTmPc, BBY, BTmPd, TSID3B, TS1D2A and TS1D2B could produce high percentage of germination.

Table 3. Conidial viability of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	Conidial viability ± SD (%) of 24-hour-suspension culture		Conidial viability ± SD (%) of 48-hour-suspension culture	
	25 °C	34 °C	25 °C	34 °C
BPcMs	70.17±4.80 ab	15.54±1.53 bcdef	77.46±1.59	26.05±2.10 hijk
BTmKt	47.77±9.63 a	16.72±2.27 bcdefg	58.38±2.75	24.75±3.98 fghijk
BTmPc	66.46±7.62 ab	26.30±1.95 hi	76.35±1.97	28.54±1.93 jkl
Bws Pantura	66.51±8.71 ab	9.09±8.32 ab	75.48±13.12	10.57±9.17 ab
BBY	73.02±4.97 ab	23.56±1.3 ghi	75.05±2.88	26.05±2.10 hijk
BTmPe	63.44±12.89 ab	22.28±8.793 fghi	70.85±4.46	25.49±7.21 ghijk
BTmMa	67.94±9.75 ab	14.26±3.90 bcde	72.68±6.60	14.26±3.91 bcd
BTmSo	74.83±6.15 ab	19.71±3.78 cdefgh	77.55±6.13	22.47±1.86 fghi
BTmSr	73.62±8.85 ab	20.99±1.89 defgh	75.47±7.67	20.99±1.89 efgh
BuBj	74.30±6.82 ab	21.64±4.26 efghi	78.69±4.45	22.92±2.76 fghij
725HaJ	66.49±10.80 ab	14.03±2.93 bcd	72.10±7.62	16.63±5.18 cde
715HhB	68.77±2.28 ab	11.20±9.71 abc	74.43±4.34	13.32±11.73 bc
BTmPd	75.19±10.56 ab	23.77±4.46 ghi	76.45±9.77	28.52±2.66 jkl
BTmTs	76.30±11.60 ab	12.40±3.79 abc	79.85±10.47	12.40±3.79 bc
BTmkbc	66.24±1.30 ab	22.51±4.88 fghi	70.43±1.53	22.51±4.88 fghi
BPcPd2	71.44±8.88 ab	7.98±7.19 a	70.42±3.09	7.98±7.19 a
BPcPd	63.60±18.14 ab	15.10±3.93 bcdef	69.81±12.77	15.10±3.93 bcd
BMkMs	65.06±5.80 ab	14.42±6.65 bcde	72.14±6.04	21.53±0.67 efgh
BTmTr	70.00±13.82 ab	15.44±1.84 bcdef	73.70±7.60	24.02±2.00 ghijk
Natural BVR#	60.74±9.62 ab	28.53±1.06 i	69.73±10.85	32.58±3.49 l
BTmGa	63.42±11.63 ab	19.89±1.29 cdefgh	67.15±8.12	19.89±1.29 defg
BLePd	48.80±6.83 a	17.17±3.87 cdefg	59.00±4.84	19.31±3.44 def
TS1D3A	79.33±6.36 b	17.75±3.90 cdefg	81.42±4.73	25.61±1.77 ghijk
TSID3B	69.49±4.96 ab	15.87±1.02 bcdef	80.23±5.33	28.72±1.60 jkl
TS1D2A	78.41±4.33 b	17.69±0.92 cdefg	81.38±1.38	28.12±5.33 ijkl
TS1D2B	70.29±4.45 ab	15.14±4.07 bcdef	80.58±7.39	29.22±4.32 kl
ANOVA F-value	2.02*	3.40*	2.03ns	5.89*
P value (0.05)	10×10^{-8}	4.6×10^{-6}	9.7×10^{-10}	9×10^{-10}
Tukey's HSD test	18.057	7.45	-	7.56

Remarks: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test

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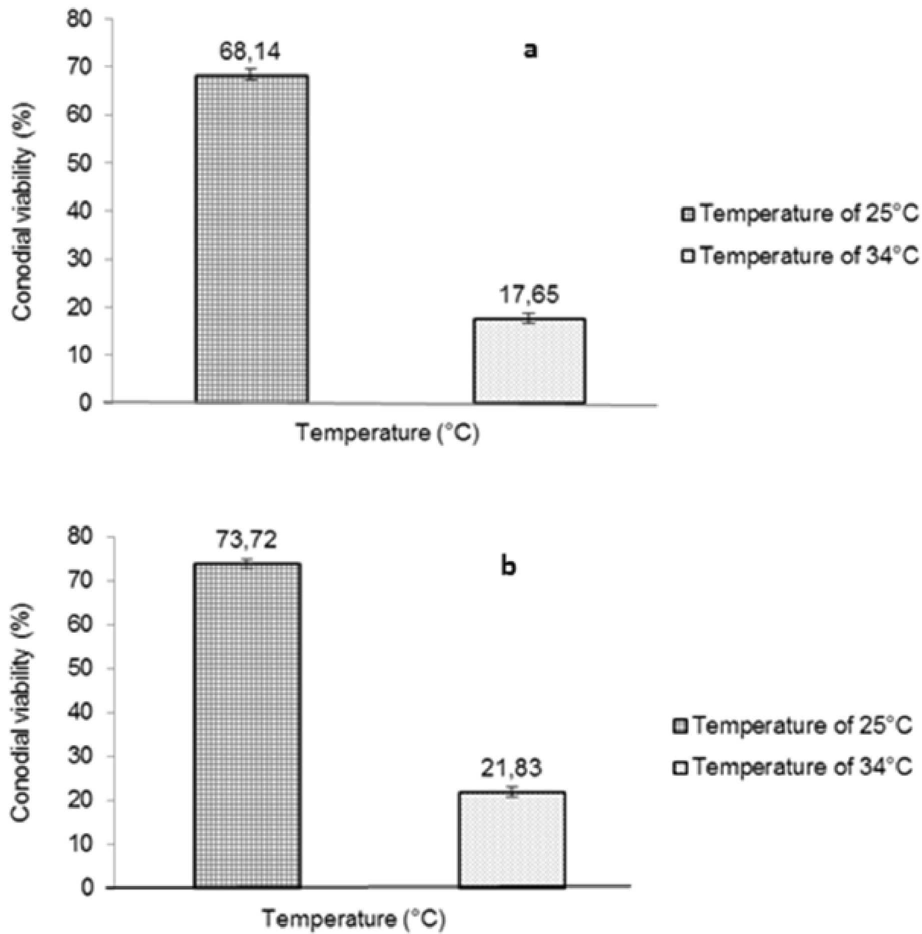

Fig. 4. Conidial viability of *Beauveria bassiana* culture incubated for 7 days at 25 and 34 °C in 24-hour (P = 0.00) (a) and 48-hour suspension culture (P = 0.00) (b)

The shape of conidial germination of *B. bassiana* in this research was similar to description of conidial germination given by the following researcher. Conidial germination showed the following signs: broken conidial wall resulting in germ tube formation which had long stretch and increased in size or diameter of conidia (Oliveira, Pauli, Mascarin, & Delalibera, 2015; Safitri, Herlinda, & Setiawan, 2018). Percentage of conidial germination was used to measure conidial viability of the fungus. Conidial viability of *B. bassiana* at 25 °C was high on TS1D3A, BTmKt and BLePd isolates. However, the high conidial viability was found on TS1D3A, commercial BVR#, BTmPc, BBY, BTmPd, TSID3B and TS1D2B isolates at 34 ºC. The conidial viability of *B. bassiana* in 24-hour suspension or 48-hour suspension culture was decreased significantly at incubation temperature of 34 °C. The ideal temperature for the conidial germination of *B. bassiana* was in the range of 25 to 27 °C, but *B. bassiana* conidia could still germinate at 32 °C (Constanski et al., 2011). Salim, Md. Rawi,

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Ahmad, & Al-Shami (2015) had stated that the conidial germination of *B. bassiana* still occurred up to 33 °C. Local isolates of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B originated from soils and insects of South Sumatra and East Java, Indonesia still had high conidial viability at 34 °C. The conidial viability of these local isolates were equivalent to those of the commercial BVR# isolate. The conidial isolates that were capable to germinate at 34 °C were rarely occurred, but only high temperature resistant isolates that had the capability to germinate. According to Salim, Md. Rawi, Ahmad, & Al-Shami (2015) only superior strains of the fungi were able to germinate at above 33 ºC. The conidia of the local isolates in this experiment that were capable to germinate at 34 ºC had potential to be developed as active ingredients of bioinsecticides to control *N. lugens* in high temperature rice ecosystem, such as wetland or lowland rice ecosystems of South Sumatra, Indonesia. According to Siaga et al. (2019) the temperature occured in wetland or lowland rice ecosystems of South Sumatra was above 30 °C.

Virulence of *Beauveria bassiana*

The symptom of *N. lugens* nymphs infected by *B. bassiana* started to appear at the second day after being exposed to the fungal conidia with doses of 1 x 10^3 conidia per cm^2. The nymphs had slow movement and finally stopped moving, whereas the healthy nymphs still actively moved on the rice stem. On the third day, some of the sick nymphs were dead and the other infected nymphs which were still alive became unable to move anymore with their legs and stylets attached on the rice stem. On the fourth and fifth days, the dead nymphs hung with their stylets still attached on the rice stem, while all their legs did not grip on the rice stem anymore. On the sixth day, the dead nymphs became hardened and stiff. On the seventh day, their bodies wrinkled, decayed with no smell and their integuments were coated with white color mycelia which gradually became brownish white to dark brown colors (Fig. 5).

Virulence of *B. bassiana* isolates against *N. lugens* nymphs was represented on the percentage of mortality and the lethal time to 50% (LT_{50}) and 95% (LT_{95}) mortality of *N. lugens* nymphs caused by *B. bassiana*. All isolates of *B. bassiana* tested in this experiment were pathogenic against the *N. lugens* nymphs. At temperature of 25 °C, mean mortality of *N. lugens* nymphs with magnitude of 96.67% was found on TS1D2B isolate, whereas the lowest mean mortality with magnitude of 20% was found on BPcPd2 isolate (Table 4).

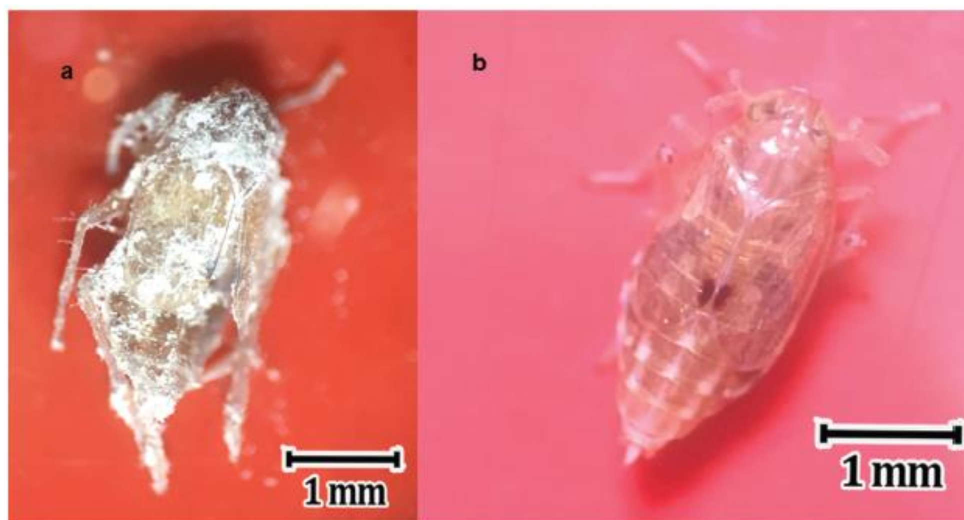

Fig. 5. *Nilaparvata lugens* infected by *Beauveria bassiana* (a) and the healthy one (b)

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Table 4. Virulence of *Beauveria bassiana* isolates culture incubated at 25 and 34 °C against *Nilaparvata lugens* nymphs

Isolate codes	Mortality of *Nilaparvata lugens* nymphs ± SD (%)	
	25 °C	34 °C
BPcMs	65.00±5.00	21.67±5.77
BTmKt	26.67±10.41	13.33±14.43
BTmPc	43.33±7.64	28.33±7.64
Bws Pantura	30.00±18.03	5.00±5.00
BBY	38.33±15.28	16.67±7.64
BTmPe	28.33±10.41	21.67±17.56
BTmMa	28.33±14.43	25.00±18.03
BTmSo	56.67±10.41	40.00±18.03
BTmSr	35.00±5.00	35.00±5.00
BuBj	45.00±21.79	26.67±28.87
725HaJ	23.33±2.89	20.00±5.00
715HhB	15.00±5.00	6.67±7.64
BTmPd	26.67±7.64	20.00±15.00
BTmTs	26.67±10.41	13.33±2.89
BTmkbc	28.33±15.28	28.33±15.28
BPcPd2	20.00±10.00	10.00±10.00
BPcPd	25.00±10.00	25.00±10.00
BMkMs	26.67±14.43	26.67±14.43
BTmTr	31.67±24.66	20.00±5.00
Natural BVR[#]	43.33±12.58	36.67±7.64
BTmGa	30.00±5.00	30.00±5.00
BLePd	26.67±11.55	23.33±5.77
TS1D3A	90.00±13.23	38.33±11.55
TSID3B	58.33±7.64	43.33±14.43
TS1D2A	81.67±20.21	41.67±15.28
TS1D2B	96.67±5.77	43.33±18.93
ANOVA F-value	0.29ns	0.16ns
P value (0.05)	0.99	1.00

Remarks: ns = not significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to HSD test.

The highest value of mean mortality of *N. lugens* nymphs at 34 °C was found on TS1D2B isolate (43.33%), wehereas the lowest value of mean mortality of *N. lugens* nymphs was found on Bws Pantura isolate (5%). However, mortality of *N. lugens* nymphs caused by *B. bassiana* was not significantly different among all isolates either at temperarture of 25 or 34 °C. The percentage of *N. lugens* mortality was significantly decreased when fungal incubation temperature was increased from 25 to 34 °C (P = 0.03) (Fig. 6). Therefore, the incubation temperature at 34 °C for 7 days was significantly decreased the virulence of some *B. basiana* isolates.

All isolates of *B. bassiana* were pathogenic against *N. lugens* nymphs. The mortality of the *N. lugens* nymphs caused by all isolates was high. However, virulence of *B. bassiana* isolates against *N. lugens* nymphs was significantly decreased if *B. bassiana* culture was incubated at 34 °C. Virulence of *B. bassiana* was decreased at 34 °C due to the decrease of conidial viability.

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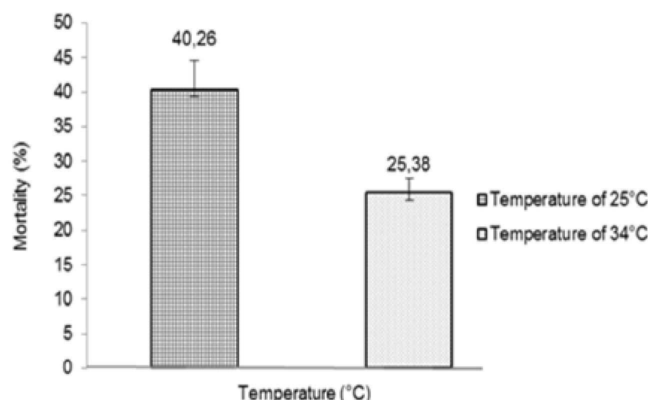

Fig. 6. Mortality of *Nilaparvata lugens* caused by *Beauveria bassiana* culture incubated at 25 and 34 °C (P = 0.003)

Virulence of *B. bassiana* was affected by the conidial viability (Ghany, 2015). The higher of the capability of conidia germinate, the higher the probability of germ tube formation of the conidia penetrate insect cuticle (Butt, Ibrahim, Ball, & Clark, 1994; Fernandes, Rangel, Moraes, Bittencourt, & Roberts, 2007). The local isolates from South Sumatra of BTmPc, BBY, BTmPd, TS1D3A, TSID3B and TS1D2B in this study still had high conidial viability at 34 °C which in turn cause the high percentage of *N. lugens* mortality. These local isolates could adapt to high temperature of 34 °C and could be chosen as candidates for biocontrol agents of *N. lugens* in wetland or lowland rice ecosystems in Indonesia.

At fungal incubation temperature of 25 °C, mean of LT_{50} values ranged from 2.24 to 5.06 days and the shortest time was found on BTmPd isolate, whereas the longest time was found on 715 HHBanyuwangi isolate (Table 5). Mean of LT_{50} values caused by *B. bassiana* incubated at 34 °C ranged from 2.92 to 10.40 days and the shortest time was found on isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. They were isolates from South Sumatra. Whereas, the longest time was found on Bws Pantura isolate. TSID3B isolate culture incubated at 25 ºC had the shortest lethal time values of 2.34 days (LT_{50}) and 7.16 days (LT_{95}). However, LT_{50} values were not significantly different among all isolates of *B. bassiana* and similar trend was also occurred on LT_{95}. Incubation temperature for *B. bassiana* culture affects LT_{50} or LT_{95} values. Mean of LT_{50} or LT_{95} values was significantly longer on *B. bassiana* incubated at 34 ºC than that of *B. bassiana* incubated at 25ºC (Fig. 7). Therefore, incubation temperature at 34 ºC for 7 days for *B. bassiana* culture could extend the lethal time to 50% and 95% mortality of *N. lugens* nymphs.

The incubation temperature at 34 °C for *B. bassiana* culture could prolong the LT_{50} and LT_{95} of *N. lugens* nymphs, but some isolates that still have short LT_{50} or LT_{95} value were consisted of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B. LT_{50} or LT_{95} value of *N. lugens* caused by *B. bassiana* was affected by conidial viability of the fungus. The lower percentage of conidial germination caused longer time for the fungus to invade the whole body of insect hosts (Fernandes, Rangel, Moraes, Bittencourt, & Roberts, 2007). In this research, the isolates of BtmMa, BtmSo, BTmSr, BTmkbc, BtmGa, BLePd and TSID3B at 34ºC causing the lethal time to 50% mortality of *N. lugens* nymphs were 3−4 days. The normal time required by conidia to kill insect host was 4−10 days (Ghany, 2015). The time required by *B. bassiana* to kill *N. lugens* nymphs in this study were shorter because the insect hosts were more sensitive than other insect host species. Conidia requires certain time to germinate on insect cuticle surface and subsequently mycelia penetrates into body cavity (Fernandes, Rangel, Moraes, Bittencourt, & Roberts, 2007). Then, the host insect will die whitin 4 days (Butt, Ibrahim, Ball, & Clark, 1994). Next, the fungus yields thousands of new spores on the dead body (Ghany, 2015).

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Table 5. LT_{50} and LT_{95} of *Nilaparvata lugens* nymphs caused by *Beauveria bassiana* isolates culture incubated at 25 and 34 °C

Isolate codes	LT_{50} (days) (95 % fiducial limits)		LT_{95} (days) (95 % fiducial limits)	
	25 °C	34 °C	25 °C	34 °C
BPcMs	2.62 (1.68 – 3.72)	4.95 (3.71 – 6.14)	7.43 (5.66 – 9.15)	11.40 (9.43 – 12.78)
BTmKt	4.18 (3.37 – 4.82)	7.48 (5.77 – 8.76)	9.00 (8.35 – 10.25)	13.93 (11.63 – 16.56)
BTmPc	3.86 (2.58 – 5.28)	4.87 (4.26 – 5.20)	8.58 (7.71 – 10.03)	11.33 (9.97 – 13.00)
Bws Pantura	4.31 (2.86 – 6.85)	10.40 (7.22 – 14.22)	9.13 (8.26 – 10.83)	16.85 (12.94 – 22.01)
BBY	2.93 (2.60 – 3.33)	5.96 (3.62 – 8.12)	7.75 (6.84 – 8.76)	12.41 (9.34 – 15.91)
BTmPe	3.88 (3.65 – 4.23)	5.92 (3.11 – 10.56)	8.69 (8.21 – 9.08)	12.37 (8.96 – 18.36)
BTmMa	2.76 (2.01 – 4.16)	2.99 (1.36 – 4.42)	7.57 (6.09 – 9.19)	9.45 (7.08 – 12.22)
BTmSo	3.31 (2.68 – 4.42)	3.09 (2.56 – 3.68)	8.13 (7.71 – 8.40)	9.55 (8.27 -11.48)
BTmSr	2.57 (1.94 – 2.95)	2.92 (2.40 – 3.60)	7.39 (6.94 – 8.26)	9.38 (8.11 – 10.56)
BuBj	2.68 (1.76 – 4.16)	6.43 (1.45 – 10.35)	7.49 (6.09 – 9.19)	12.89 (7.17 – 18.14)
725HaJ	3.19 (2.73 – 3.76)	5.65 (4.35 – 7.04)	8.00 (7.05 – 8.79)	12.00 (11.41 – 12.45)
715HhB	5.06 (2.98 – 6.76)	9.00 (6.92 – 10.47)	9.88 (6.96 – 12.19)	15.46 (12.77 – 17.42)
BTmPd	2.24 (1.59 – 3.24)	5.02 (2.73 – 8.85)	7.06 (5.88 – 8.67)	11.47 (8.44 – 16.65)
BTmTs	3.93 (3.07 – 4.98)	6.55 (5.44 – 8.12)	8.75 (7.05 – 10.40)	13.01 (11.15 – 15.91)
BTmkbc	4.61 (3.24 – 5.32)	3.31 (1.21 – 6.13)	9.13 (7.77 – 10.31)	9.76 (6.92 – 13.93)
BPcPd2	4.21 (2.73 – 6.82)	8.15 (2.75 – 14.22)	9.02 (7.05 – 11.85)	14.61 (8.46 – 22.01)
BPcPd	4.39 (3.09 – 5.65)	4.37 (3.09 – 6.51)	9.20 (8.40 – 10.68)	10.91 (8.80 – 12.64)
BMkMs	4.19 (2.66 – 7.09)	3.95 (2.23 – 6.65)	9.01 (6.64 – 12.12)	10.74 (7.95 – 12.51)
BTmTr	3.82 (2.91 – 4.65)	4.66 (3.97 – 5.09)	8.64 (6.89 – 10.08)	11.12 (9.82 – 12.72)
Natural BVR#	4.12 (3.37 – 5.44)	4.06 (3.65 – 4.65)	8.94 (7.35 – 10.47)	10.51 (9.71 – 11.45)
BTmGa	3.68 (2.93 – 4.36)	3.96 (2.94 – 4.66)	8.50 (8.35 – 8.79)	10.41 (8.66 – 12.08)
BLePd	3.90 (3.33 – 4.59)	3.79 (3.50 – 3.97)	8.72 (8.36 – 9.22)	10.25 (9.62 – 11.29)
TS1D3A	2.99 (2.46 – 3.55)	4.17 (4.07 – 4.32)	7.80 (6.94 – 8.98)	10.63 (9.79 – 11.92)
TSID3B	2.34 (1.63 – 3.72)	3.92 (3.23 – 4.91)	7.16 (5.61 – 9.15)	10.38 (9.48 – 11.02)
TS1D2A	2.41 (1.19 – 3.10)	5.80 (4.17 – 7.26)	7.23 (6.62 – 7.97)	12.26 (9.88 – 13.78)
TS1D2B	4.19 (3.05 – 5.28)	4.35 (3.48 – 4.82)	9.01 (8.23 – 10.31)	10.67 (10.13 -11.28)
ANOVA F-value	0.84ns	2.08ns	0.84ns	1.21ns
P value (0.05)	0.68	0.01	0.68	0.28

Remarks: ns = not significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at $P < 0.05$ according to HSD test.

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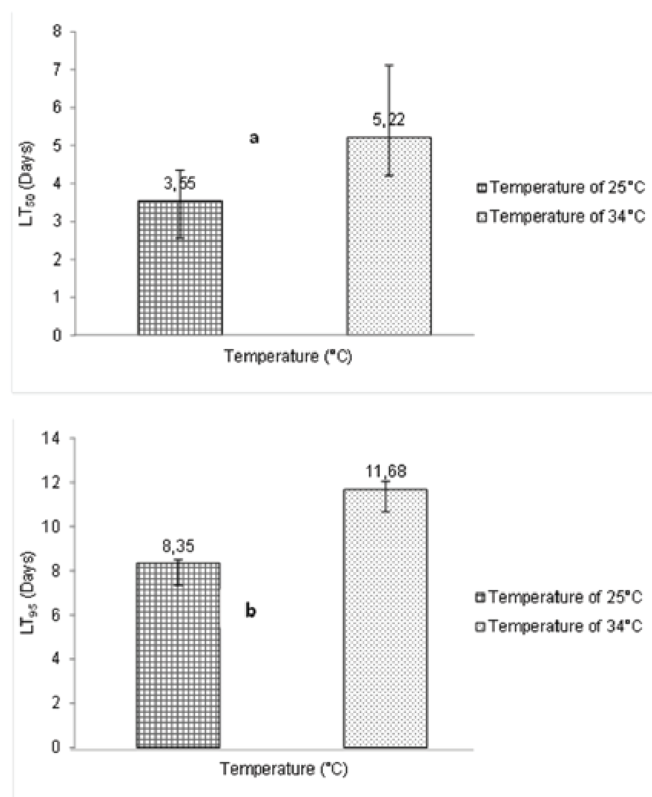

Fig. 7. LT50 (P = 0.00) (a) and LT95 (P = 0.00) (b) caused by *Beauveria bassiana* culture incubated at 25 and 34 °C against Nilaparvata lugens

CONCLUSION

It can be concluded from this study that at germination temperature of 34 °C, some isolates of *B. bassiana* originate from soils or insects, especially from South Sumatra, could produce high conidial density and viability as well as high virulent against *N. lugens* nymphs. The importance of this finding showed that some isolates were still virulent although their culture were incubated at 34 °C for 7 days. Therefore, the isolates can be used as promising candidates for biocontrol for *N. lugens* on rice planted in tropical ecosystem, such as tidal lowland and lowland swamp ecosystems in Indonesia.

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