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Molecular identification of three entomopathogenic fungi infecting the brown plant hopper pest in Indonesia

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Abstract

Background: Brown plant hopper *(Nilaparvata lugens* Stal.) a very damaging pest to rice crops. One of the efforts to control it is the use of entomopathogenic fungi (EPF). Three fungal local isolates found in Indonesia were effective in controlling the brown plant hopper pest. This study aimed to molecularly identify the 3 fungal isolates. Molecular identification is very important to get the exact identity of these fungi. The accuracy of EPF identification will greatly determine the success of control. Molecular identification is based on a partial genetic analysis of the internal transcribed spacer (ITS) locus of ribosomal fungal DNA.

Result: Morphology of the local isolates named J22 and J60 were identified as *Paecilomyces* sp., while the isolate J34 was identified as *Beauveria* sp. The results of molecular identification of the isolates J22 and J60 were identified as the fungi *Lecanicillium saksenae* and *Simplicillium* sp., while isolate J34 was identified as *Myrothecium* sp. The results of literature search showed that the 3 fungi have never been previously reported to infect the brown plant hopper.

Conclusion: In Indonesia, 3 types of EPF, namely *L. saksenae*, *Simplicillium* sp., and *Myrothecium* sp., were found having the potential to control the brown plant hopper pest.

Keywords: Entomopathogenic fungus, *Lecanicillium saksenae*, Molecular identification, *Myrothecium* sp., *Nilaparvata lugens*, *Simplicillium* sp., Brown plant hopper

Background

Brown planthopper (BPH) *Nilaparvata lugens* is a major insect pest of rice that causes 20–80% yield loss through direct and indirect damage. The typical damage caused by BPH is drying of plants as if burning (hopperburn) (Balachiranjeevi et al. 2019). BPH can also transmit grassy stunt and ragged stunt viruses (Helina et al. 2019).

The frequency of BPH infestation is increasing frequently in developing Asian countries due to the killing of its natural enemies because of the use of synthetic chemical insecticides (Minarni et al. 2018). Entomopathogenic fungi (EPF) are fungi that can infect and kill insects (Litwin et al. 2020). The EPF that have been widely researched and known to be effective for controlling BPH pests are *B. bassiana* (Sumikarsih et al. 2019) and *Metarhizium* sp. (Chinniah et al. 2016). However, in their implementation in the field, the use of EPF to control BPH pests still has many weaknesses. After application in the field, insect pathogens are exposed to various abiotic stresses such as temperature and humidity (Hsia et al. 2014), UV radiation (Shafighi et al. 2014), and edaphic factors (Klingen et al. 2015).

In addition to biotic stress, the effectiveness of EPF in controlling insect pests is influenced by the diversity of varieties or strains or types of them. EPF have large genetic variations among different isolates. The pathogenicity, virulence, enzymatic characteristics, and DNA also varied among different isolates of different insects. The origin of the isolate affects the virulence diversity of the

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fungus against the host insect, due to the type or race or strain of the fungus (Chen et al. 2017a, b).

The results of previous studies have reported 3 effective fungal isolates to control the brown plant hopper pest. The 3 isolates caused 70–80% mortality within 3.43–4.87 days. The 3 isolates were Pasir Kulon (J22), Cipete (J34), and Papringan (J60). According to morphological characteristics, isolates J22 (Pasir Kulon) and J60 (Papringan) were identified as *Paecilomyces* sp., while J34 (Cipete) isolate was identified as *Beauveria* sp. (Minarni et al. 2020).

Accuracy of identification is very important in the use of EPF for insect pest control. Identification based on morphological characters cannot be used to distinguish fungi to the species level so it is necessary to identify them molecularly (Imoulan et al. 2017). This research aimed to precisely identify the 3 previously mentioned EPF isolates that attack the brown plant hoppers.

Methods

Identification process

Fungal isolates J22 (Pasir Kulon), J34 (Cipete), and J60 (Papringan) were identified molecularly based on a partial genetic analysis on the internal transcribed spacer (ITS) locus of ribosomal DNA of fungi. Fungal isolates that will be identified previously were grown in potato dextrose broth (PDB) liquid media. After being incubated for 72 h, the fungal mycelia were harvested, using sterile filter paper and washed with sterile distilled water. The fungal mycelia were crushed in a sterile mortar by a sterile grinder and liquid nitrogen was added. Half a gram of dry fungal biomass was transferred to a 1.5-ml micro-tube containing 600 μl of cetyl trimethylammonium bromide (CTAB) buffer solution. Afterwards, the tube was shaken out and incubated at 65 °C for 30 min, then incubated in ice for 5 min. A mixture of chloroform and isoamyl alcohol with a ratio of 24:1 of 600 μl was added to the tube. The tubes were then centrifuged at 4 °C for 10 min at a speed of 25,000×*g*. The supernatant was transferred to a new tube and added with 0.1× volume of 2M NaOAc pH 5.2 and 3× volume of ethanol then incubated at − 20 °C for 2 h.

Fungal DNA pellets were obtained by centrifugation at 25,000×*g* at 4 °C for 25 min. The fungal DNA pellets were washed by 500 μl of 70% ethanol, then centrifuged at 25,000×*g* at 4 °C for 5 min. The fungal DNA pellets were dried in an airtight chamber for 5 min, then dissolved in 0.2× volume of RNAse and 30 μl of sterile TE (TrisHCl 10 mM, pH 7.4, EDTA 1 mM) buffer and then incubated at 37 °C for 10 min and 70 °C for 10 min.

Extraction of fungal DNA was done using Nucleon PhytoPure reagent kit (Amersham LIFE SCIENCE, USA). PCR amplification was at ITS, using ITS Primer 4: 5′-TCC TCC GCT TAT TGA TAT GC-3′ and ITS Primer 5: 5′-GGA AGT AAA AGT CGT AAC AAG G-3′ (White et al. 1990). DNA amplification was carried out by making a volume of 30 μl containing 10.5 μl of alkaline free water, 15 μl 2× PCR mastermix (Promega), 0.75 μl and 10 pmol respectively of primer ITS 4 and ITS 5 and 3 μl (about 250 ng/μl) DNA template. The amplification reaction was carried out in 35 cycles as follows: pre-denaturation at 95 °C for 15 min, denaturation at 95 °C for 30 min, heating (annealing) at 55 °C for 30 s, lengthening at 72 °C for 1.5 min, re-extension at 72 °C for 5 min. and lastly stored at 25 °C for 10 min.

Purification of PCR products was carried out by using Polyethilen Glycol (PEG) precipitation method (Hiraishi et al. 1995) and continued with a sequencing cycle. The results of sequencing cycle were purified again, using the ethanol purification method. Analysis of nitrogen base sequence readings was done using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The raw data resulting from the sequencing was then trimmed and assembled, using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequence data that was assembled was then carried out in BLAST with genomic data that was registered at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) to determine taxon or species that have the greatest homology/similarity and molecularly.

Results

Morphological identification

Fungal isolates, isolated from brown plant hoppers, were infected by EPF. Fungi were purified and cultured on potato dextrose agar (PDA) media. The results of the observation on morphological characteristics, the isolates J22 (Pasir Kulon) and J60 (Papringan) were identified as

Table 1 Morphological characteristics of entomopathogenic fungi J22, J34, and J60 isolates

Isolate	Color and shape of the colony	Conidial form	Conidial color	Genus	References
J22 and J60	Round, flat, white which then turns to be creamy in old age	fusiform, sometimes cylindrical, and smooth walled	Hyaline	*Paecilomyces* (Figs 1 and 3)	Dong et al. (2016), Nguyen et al. (2017)
J34	White, the edges are pale yellow and the base color is white, round shape, and widened growth	oval slightly rounded, stick to the ends and sides of the conidiophores (branches), have long crossed hyphae, and conidial growth clustered.	Hyaline	*Beauveria* (Fig. 2)	Rosmini and Lasmini (2010), Nuraida and Hasyim (2009)

Paecilomyces sp. while J34 (Cipete) isolate was identified as *Beauveria* sp. (Minarni et al. 2020). The morphological characters of each EPF isolate (J22, J34 and J60) are presented in (Table 1 and Figs. 1, 2, and 3).

Molecular identification

The results of the ITS rDNA sequencing of fungal isolates J22, J34, and J60 are as follows:

1. ITS rDNA isolate sequence
   (a) Pasir Kulon_ITS4

1	TCACGT TCAG	AAAGTGGG GT	GTTTTACG GC	GTGGCCAC GT	CGGGGTTC CG
51	GTGCGAGG TT	GGATTACT AC	GCAGAGGT CG	CCGCGG ACGG	GCCGCCAC TC
101	CATTTCGG GG	CCGGCGGT AT	GCTGCCGG TC	CCCAACGC CG	ATTTCC CCAA
151	AGGGAAGT CG	AGGGTTGA AA	TGACGC TCGA	ACAGGCAT GC	CCGCCAGA AT
201	GCTGGCGG GC	GCAATGTG CG	TCAAAGAT TC	GATGATTC AC	TGAATTCT GC
251	AATTCACA TT	ACTTATCG CA	TTTCGCTG CG	TTCTTCAT CG	ATGCCAGA AC
301	CAAGAGAT CC	GTTGTTGA AA	GTTTTTGA TTC	ATTTGTTT TG	CCTTGCGG CG
351	GATTCAGA AG	ATACTC ATGA	TACAAA AGAG	TTTGGTGG TC	TCCGGCGG CC
401	GCCTGAGT CC	GGGCCG CGGG	CGGCGC TAGG	CCGTCCGG AC	GCCGGG GCGA
451	GTCCGC CGAA	GCAACATC TT	GGTATGTT CA	CATAAGGG TT	TGGGAGTT GT
501	AAACTCTG TA	ATGATCCC TC	CGCTGGTT CA	CCAACGGA GA	CCTTGTTAC

(b) Pasir Kulon_ITS5

1	GTTGCT TCGG	CGGACTCG CC	CCGGCGTC CG	GACGGC CTAG	CGCCGCCC GC
51	GGCCCGGA CT	CAGGCGGC CG	CCGGAGAC CA	CCAAACTC TT	TTGTATCA TG
101	AGTATCTT CT	GAATCCGC CG	CAAGGCAA AA	CAAATGAA TC	AAAACTTT CA
151	ACAACGGA TC	TCTTGGTT CT	GGCATCGA TG	AAGAAC GCAG	CGAAAT GCGA
201	TAAGTAAT GT	GAATTG CAGA	ATTCAG TGAA	TCATCGAA TC	TTTGAACG CA
251	CATTGCGC CC	GCCAGCAT TC	TGGCGGGC AT	GCCTGT TCGA	GCGTCATT TC

Molecular identification *(Continued)*

301	AACCCTCG AC	TTCCCT TTGG	GGAATTCG GC	GTTGGGGG AC	CGGCAGCA TA
351	CCGCCGGC CC	CGAAATGG AG	TGGCGGCC CG	TCCGCG GCGA	CCTCTGCG TA
401	GTAATCCA AC	CTCGCA CCGG	AACCCCGA CG	TGGCCACG CC	GTAAAACA CC
451	CCACTTTC TG	AACGTTGA CC	TCGGAT CAGG	TAGGAATA CC	CGCTGAAC TT
501	AA				

(c) Contig-PasirKulon

1	GTAACAAG GT	CTCCGTTG GT	GAACCA GCGG	AGGGATCA TT	ACAGAGTT TA
51	CAACTC CCAA	ACCCTTAT GT	GAACATAC CA	AGATGTTG CT	TCGGCGGA CT
101	CGCCCCGG CG	TCCGGACG GC	CTAGCGCC GC	CCGCGGCC CG	GACTCAGG CG
151	GCCGCCGG AG	ACCACCAA AC	TCTTTTGT AT	CATGAGTA TC	TTCTGAAT CC
201	GCCGCAAG GC	AAAACAAA TG	AATCAAAA CT	TTCAACAA CG	GATCTC TTGG
251	TTCTGGCA TC	GATGAAGA AC	GCAGCGAA AT	GCGATAAG TA	ATGTGAAT TG
301	CAGAAT TCAG	TGAATCAT CG	AATCTT TGAA	CGCACATT GC	GCCCGCCA GC
351	ATTCTG GCGG	GCATGCCT GT	TCGAGCGT CA	TTTCAACC CT	CGACTTCC CT
401	TTGGGGAA AT	CGGCGT TGGG	GGACCG GCAG	CATACCGC CG	GCCCCGAA AT
451	GGAGTG GCGG	CCCGTCCG CG	GCGACCTC TG	CGTAGTAA TC	CAACCTCG CA
501	CCGGAACC CC	GACGTGGC CA	CGCCGTAA AA	CACCCCAC TT	TCTGAACG TT
551	GACCTCGG AT	CAGGTAGG AA	TACCCG CTGA	ACTTAA	

(d) Cipete_ITS4

1	CGGCAGGG GC	TCCGTCCG CT	TCTCCCTA TG	CGGAATAT CA	CTACTTCC GC
51	AGGGGAGG CC	ACGACGGG TC	CGCCAC TAGA	TTTAGGGG CC	GGCCGTCC CT
101	CGCGGGCG CT	GGCCGATC CC	CAACACCA CG	CCCTAGGG GC	ATGAGGGT TG

Molecular identification *(Continued)*

151	AAATGACG CT	CAGACAGG CA	TGCCCG CCAG	AATACTGG CG	GGCGCAAT GT
201	GCGTTCAA AG	ATTCGATG AT	TCACTGAA TT	CTGCAATT CA	CATTACTT TT
251	CGCATTTC GC	TGCGTTCT TC	ATCGATGC CA	GAACCAAG AG	ATCCGTTG TT
301	GAAAGTTT TT	ATTTATTT GT	AAAAACGA CT	CAGAAGAT TC	TCAGTAAA AC
351	AAGAGT TAAG	GTCCCCCG GC	GGCCGC CTGG	ATCCGGGG CA	CGCAAGGC GC
401	CCGGGGCG AT	CCGCCGAA GC	AACGATAG GT	ATGTTCAC AT	GGGTTTGG GA
451	GTTGTAAA CT	CGGTAATG AT	CCCTCCGC TG	GTTCACCA AC	GGA

(e) Cipete_ITS5

1	TCGTTGCT TC	GGCGGATC GC	CCCGGGCG CC	TTTGCGTG CC	CCGGAT CCAG
51	GCGGCCGC CG	GGGGACCT TA	ACTCTTGT TT	TTACTGAG AA	TCTTCTGA GT
101	CGTTTTTA CA	AATAAA TAAA	AACTTTCA AC	AACGGATC TC	TTGGTT CTGG
151	CATCGA TGAA	GAACGCAG CG	AAATGCGA AA	AGTAAT GTGA	ATTGCAGA AT
201	TCAGTGAA TC	ATCGAATC TT	TGAACGCA CA	TTGCGCCC GC	CAGTATTC TG
251	GCGGGCAT GC	CTGTCTGA GC	GTCATT TCAA	CCCTCATG CC	CCTAGGGC GT
301	GGTGTTGG GG	ATCGGCCA GC	GCCCGCGA GG	GACGGCCG GC	CCCTAAAT CT
351	AGTGGCGG AC	CCGTCGTG GC	CTCCCCTG CG	AAGTAGTG AT	ATTCCGCA TA
401	GGAGAGCG AC	GAGCCCCT GC	CGTTAAAC CC	CCAACTTT CT	CAGGTTGA CC
451	TCAGAT CAGG	TAGGAATA CC	CGCTGAAC TT	A	

(f) Contig-Cipete

1	TCCGTTGG TG	AACCAG CGGA	GGGATCAT TA	CCGAGTTT AC	AACTCC CAAA
51	CCCATG TGAA	CATACCTA TC	GTTGCT TCGG	CGGATCGC CC	CGGGCGCC TT
101	TGCGTGCC	GGATCCAG	GGCCGC	GGACCTTA	TCTTGTTTTT

Molecular identification *(Continued)*

	CC	GC	CGGG	AC	
151	ACTGAGAA TC	TTCTGAGT CG	TTTTTA CAAA	TAAATAAA AA	CTTTCA ACAA
201	CGGATCTC TT	GGTTCTGG CA	TCGATGAA GA	ACGCAG CGAA	ATGCGA AAAG
251	TAATGTGA AT	TGCAGAAT TC	AGTGAATC AT	CGAATCTT TG	AACGCACA TT
301	GCGCCCGC CA	GTATTCTG GC	GGGCATGC CT	GTCTGAGC GT	CATTTCAA CC
351	CTCATGCC CC	TAGGGC GTGG	TGTTGGGG AT	CGGCCAGC GC	CCGCGA GGGA
401	CGGCCGGC CC	CTAAAT CTAG	TGGCGGAC CC	GTCGTGGC CT	CCCCTG CGGA
451	AGTAGTGA TA	TTCCGC ATAG	GGAGAA GCGG	ACGGAGCC CC	TGCCGT TAAA
501	CCCCCAAC TT	TCTCAGGT TG	ACCTCAGA TC	AGGTAGGA AT	ACCCGC TGAA
551	CTTAA				

(g) Papringan_ITS4

1	TAGTTGGG TG	TTTTACGG CG	TGGCCGCT TC	GATTTTCC CA	GTGCGAGG TA
51	AGTTACTA CG	CAGAGGTC GC	CTCGAAGG GC	CGCCAC TGAA	TTTCGGGG GC
101	GGCGTCCC AC	GCCCGGAG GC	GCGGGGCA GT	CTGCCGGT CC	CCAACA CCGG
151	GCCGTCTT CC	GAAGAA TCGG	GCCCGAGG GT	TGAAATGA CG	CTCGAA CAGG
201	CATGCCCG CC	AGAATG CTGG	CGGGCGCA AT	GTGCGT TCAA	AGATTCGA TG
251	ATTCAC TGAA	TTCTGCAA TT	CACATTAC TT	ATCGCATT TC	GCTGCGTT CT
301	TCATCGAT GC	CAGAAC CAAG	AGATCCGT TG	TTGAAAGT TT	TGATTCAT TT
351	GTTTTTTG CC	TTTCGGCC AC	TCAGATAA TG	CTGTAAAA AC	AATAAGAG TT
401	TGATACCC CC	GGCAGCGC CG	GAGCGCCG CC	GAAGCA ACAA	GTGGTAAG TT
451	CACATAGG GT	TTGGGAGT TG	AATAAACT CG	ATAATGAT CC	CTCCGCTG GT
501	TCACCA ACGG	A			

(h) Papringan_ITS5

1	CCACTTGTTG	CTTCGGCGGC	GCTCCGGCGC	TGCCGGGGGT	ATCAAACTCT
51	TATTGTTTTT	ACAGCATTAT	CTGAGTGGCC	GAAAGGCAAA	AAACAAATGA
101	ATCAAAACTT	TCAACAACGG	ATCTCTTGGT	TCTGGCATCG	ATGAAGAACG
151	CAGCGAAATG	CGATAAGTAA	TGTGAATTGC	AGAATTCAGT	GAATCATCGA
201	ATCTTTGAAC	GCACATTGCG	CCCGCCAGCA	TTCTGGCGGG	CATGCCTGTT
251	CGAGCGTCAT	TTCAACCCTC	GGGCCCGATT	CTTCGGAAGA	CGGCCCGGTG
301	TTGGGGACCG	GCAGACTGCC	CCGCGCCTCC	GGGCGTGGGA	CGCCGCCCCC
351	GAAATTCAGT	GGCGGCCCTT	CGAGGCGACC	TCTGCGTAGT	AACTTACCTC
401	GCACTGGGAA	AATCGAAGCG	GCCACGCCGT	AAAACACCCA	ACTATTTTAA
451	GGTTGACCTC	GAATCAGGTA	GGACTACCCG	CTGAACTTAA	

(i) Contig-Papringan

1	TCCGTTGGTG	AACCAGCGGA	GGGATCATTA	TCGAGTTTAT	TCAACTCCCA
51	AACCCTATGT	GAACTTACCA	CTTGTTGCTT	CGCGGGCGCT	CCGGCGCTGC
101	CGGGGGTATC	AAACTCTTAT	TGTTTTTACA	GCATTATCTG	AGTGGCCGAA
151	AGGCAAAAAA	CAAATGAATC	AAAACTTTCA	ACAACGGATC	TCTTGGTTCT
201	GGCATCGATG	AAGAACGCAG	CGAAATGCGA	TAAGTAATGT	GAATTGCAGA
251	ATTCAGTGAA	TCATCGAATC	TTTGAACGCA	CATTGCGCCC	GCCAGCATTC
301	TGGCGGGCAT	GCCTGTTCGA	GCGTCATTTC	AACCCTCGGG	CCCGATTCTT
351	CGGAAGACGG	CCCGGTGTTG	GGGACCGGCA	GACTGCCCCG	CGCCTCCGGG
401	CGTGGGACGC	CGCCCCCGAA	ATTCAGTGGC	GGCCCTTCGA	GGCGACCTCT
451	GCGTAGTAAC	TTACCTCGCA	CTGGGAAAAT	CGAAGCGGCC	ACGCCGTAAA
501	ACACCCAACT	ATTTTAAGGT	TGACCTCGAA	TCAGGTAGGA	CTACCCGCTG
551	AACTTAA				

Discussion

Based on the results of the sequences, isolate J22 showed (99.83%) similarity to the *L. saksenae* strains GFRS14 and *L. saksenae* isolate Ecu121. Isolate J35 had a similarity with the sequences *Myrothecium* sp. F129 and *Myrothecium* sp. 1 TMS-2011 amounted to 98.82 and 98.93%, while isolate J60 had 99.10% similarities to the sequence *Simplicillium* sp. LCM 845.01 and 98.92% with *Simplicillium* sp. KYK00024 sequence (Table 2).

EPF isolates that showed high phylogenetic relationship and had a similarity value of 28S rDNA sequence of more than 99% with the reference species that could be expressed as one species. Ribosomal DNA sequences are used to identify and determine the phylogenetic relationships of organisms to taxa species (Bich et al. 2021). Based on the concept of phylogenetic species, it is stated that an organism is in one species when the difference in DNA sequences is between 0.2 and 1% (Shenoy et al. 2007). According to Henry et al. (2000) isolates, which have a similarity value of 100% can be stated as the same strain and a similarity value of 99% is stated as the same species, while the similarity value of 89–99% belongs to the same genus.

The similarity between 99 and 100% indicated that isolates J22, J34, and J60 each had the same chromosome number, genome size, and gene function as *L. saksenae* strain GFRS14 and *L. saksenae* strains isolate Ecu121, *Myrothecium* sp. F129, and *Myrothecium* sp. 1 TMS-2011 and *Simplicillium* sp. LCM 845.01 and *Simplicillium* sp. KYK00024, respectively.

The identification results based on morphological characters turned out to be different from molecular identification. Accuracy of identification is very important in the use of EPF for insect pest control. Identification based on morphological characters cannot be used as a definite reference. The genera *Lecanicillium, Simplicillium, Beauveria*, and *Isaria* have similar morphological characters, so that molecular identification is needed to determine the species certainty of EPF found in Banyumas Regency, Central Java Province, Indonesia. According to Lim et al. (2014) of the genus *Lecanicillium, Simplicillium* (both previously *Verticillium* spp.), *Beauveria* and *Isaria* belong to family Cordycipitaceae. According to Chen et al. (2016), the genus *Myrothecium* belongs to family Stachybotryaceae and has a worldwide distribution. Species in this genus were previously classified based on the asexual morphology, especially the characters of conidia and conidiophores. Morphology-based identification alone is imprecise because there are few characters to distinguish between species in the genus and, therefore, molecular sequence data are important in species identification.

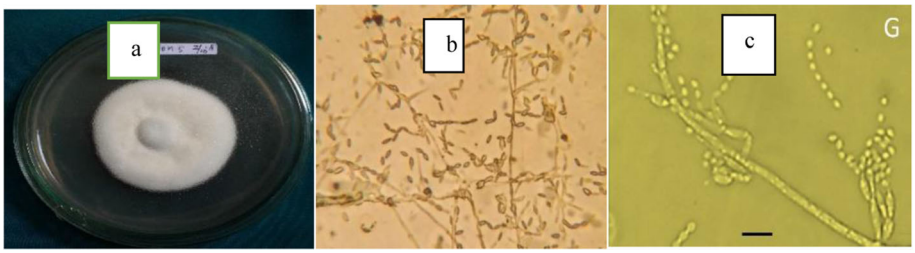

Fig. 1 **a** Colony of 8 days old Pasir Kulon (J22) isolate. **b** *Paecilomyces* sp. conidia (Minarni et al. 2020). **c** *Paecilomyces lilacinus* conidia (Dong et al. 2016)

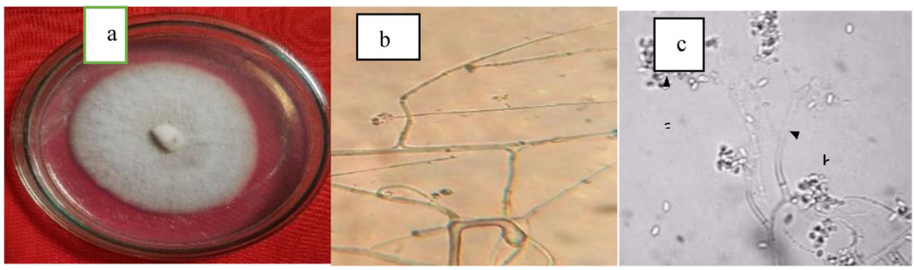

Fig. 2 **a** Colony of 8 days old Cipete (J34) isolate. **b** *Beauveria* sp. conidia (Minarni et al. 2020). **c** *Beauveria bassiana* conidia (Nuraida and Hasyim 2009)

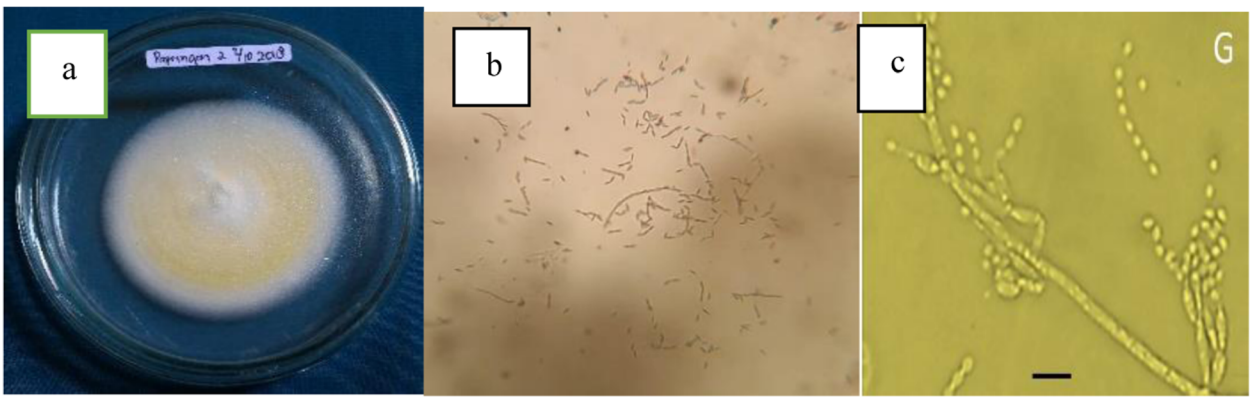

Fig. 3 **a** Pure cultures of 8 days old Papringan isolate (J60). **b** *Paecilomyces* sp. conidia (Minarni et al. 2020). **c** *Paecilomyces javanicus* conidia (Dong et al. 2016)

Table 2 Results of the nearest fungi taxon BLAST homology ITS1, 5.8S, and ITS2 of rDNA in NCBI (https://www.ncbi.nlm.nih.gov/)

Isolate	Type	No accession / host	DNAsize (bp)	Similarity percentage (%)	Query coverage (%)	Totalscore
J 22 (Pasir Kulon); No. accession/host/size, MW531463/*Nilaparvata lugens* Stal./585 bp	*Lecanicillium saksenae* strain GFRS14	MT447482/*Lycium barbarum* L	585	99.83	100	1075
	Lecanicillium saksenae isolate Ecu121	KF472156/*Coccoloba uvifera*	585	99.83	100	1075
J 34 (Cipete); No. accession/host/size, MW531464/*Nilaparvata lugens* Stal/555 bp	*Myrothecium* sp. F129	KM979797/*Glycine max* cultivar Monarca	549	98.82	100	549
	Myrothecium sp. 1 TMS-2011	HQ631058/*Saccharum officinarum*	549	98.92	100	549
J60 (Papringan); No. accession/host/size, MW531465/*Nilaparvata lugens* Stal/558 bp	*Simplicillium* sp. LCM 845.01	*MF495400/Terminalia* sp.	552	99.10	100	1002
	Simplicillium sp.KYK00024	AB378539/Acari	551	98.92	100	996

Simplicillium sp. is one of the dominant genera of symbiont fungi in unfertilized brown planthopper eggs. The other 3 genera are *Microdochium, Fusarium*, and *Cladosporium* (Shentu et al. 2020). One of the species of the genus *Simplicillium* is *S. lanosoniveum*. The fungi belong to this genus are known as mycoparasites. However, silkworms (*Bombyx mori*) inoculated with the fungus isolate *S. Lanosoniveum*, died during the larval or pupal stage, as shown by the EPF, *B. bassiana*. The first report on the entomopathogenicity of *S. lanosoniveum* and demonstrated its potential for use in insect biological control was recorded by Lim et al. (2014). The fungus *S. lanosoniveum* was able to cause mortality of *Hysteroneura setariae* ticks on Plum plants by 86.33% (Chen et al. 2017a, b). Chen et al. (2019) found 3 new species, namely *Simplicillium cicadellidae*, *S. formicidae*, and *S. lepidopterorum.* So far, there are limited reports of the fungus *Simplicillium* sp. being isolated from insects infected with the fungus.

The fungus *L. lecanii* effectively controlled brown plant hoppers with a density of 10^{10} conidia/ml, where the mortality value of (78.33%) and a time of death at 5.81 day after treatment occurred (Khoiroh et al. 2014). *L. lecanii* can cause more than 50% of brown planthopper mortality within 14 days after treatment (Atta et al. 2020), whereas according to Shaikh and Pandurang (2015), this fungus is less effective in controlling this pest. Sankar and Rani (2018) have found a new *Lecanicillium* isolate, namely *L. saksenae*, which can control stink bug (*Leptocorisa acuta*). This fungus can kill 100% of *L. acuta* nymphs and imago at 72 h after treatment at conidia densities 10^7 and 10^8.

Myrothecium verrucaria has a high activity against extracellular insect cuticles and produces chitinase, proteinase, and lipase (Vidhate et al. 2015).

Based on the literature search, the 3 fungi *Simplicillium* sp., *L. saksenae*, and *Myrothecium* sp. have never been reported to infect brown plant hopper. Data obtained from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), also showed that these 3 fungi were not obtained from insect pests (Table 2). The results of this study revealed 3 types of new EPF that had the potential to be developed as control agents for brown plant hopper pests.

Conclusion

The results of molecular identification showed that the isolates J22, J34, and J 60 were fungi from *L. saksenae, Myrothecium* sp., and *Simplicillium* sp., respectively. The results of literature search showed that these 3 fungi had never been reported to infect brown plant hopper. So that the results of this study can be considered new finding of EPF as biological agents of the control brown plant hopper pests.

Abbreviations

BPH: Brown plant hopper; BLAST: Basic Local Alignment Search Tool; CTAB: Cetyl trimethylammonium bromide; DNA: Deoxyribonucleic acid; ITS: Internal transcribed spacer; PCR: Polymerase chain reaction; PDA: Potato dextrose agar; PDB: Potato dextrose broth; PEG: Polyethilen Glycol; UV: Ultraviolet

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Authors' contributions

EWM performed the experiments on bioassay and analyzed the data. The manuscript was prepared by EWM, LS, AS, and R. All the authors read and approved the manuscript.

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