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Aspergillus oryzae and *Beauveria bassiana* as entomopathogenic fungi of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) infesting corn in Lampung, Indonesia

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Abstract

Background: *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is an important pest causing severe damage to many cultivating plants such as corn worldwide, including Indonesia. This study was performed to obtain and identify entomopathogenic fungi (EPF) of *S. litura* collected from corn fields in 4 corn producing regions of Lampung, Indonesia, as well as to investigate the damage caused by this pest on corn in Lampung Province.

Results: Three corn fields in each region were selected for collecting soil samples. Soil samples were collected from 5 corn plant rhizospheres, at each field in six months of survey. Ten larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) were laid on each soil sample as a bait, covered with a filter paper and incubated at room temperature. The emerging fungi from *T. molitor* cadaver were transferred onto Potato Dextrose Agar (PDA) medium and incubated for 7 days at room temperature. Pathogenicity test was determined against 3rd instar of *S. litura* larvae. Identification was performed based on the sequence of Internal Transcribed Spacer (ITS) Region. Observations on the corn damage caused by *S. litura* were conducted at all corn producing areas in Lampung. Twelve fungal isolates were obtained causing 0–75% of mortality of *S. litura*. Four fungal isolates (NKPT, SKHJ, SDHJ and RAHJ), which caused mortality more than 20%, were further identified. One isolate (NKPT) was confirmed as *Beauveria bassiana* and the other 3 isolates (SKHJ, SDHJ and RAHJ) were *Aspergillus oryzae*. *S. litura* generally caused slight damages to the corn which was found in every observation year performed during 2010–2019. Medium plant damage was observed in 2010–2012 and 2018–2019, severe damage was found in 2011 and crop failure was recorded in 2018.

Conclusions: *Aspergillus oryzae* and *B. bassiana* were the EPF recorded infecting *S. litura* in corn in Lampung Province. This was the first report on the isolates of *A. oryzae* as EPF of *S. litura* in Indonesia. Slight damages with *S. litura* were always recorded in every observation year but not for those of medium and severe damages and crop failure.

Keywords: Corn, *Spodoptera litura*, Entomopathogenic fungi, *Tenebrio molitor*, ITS region, Pathogenicity

Background

The tobacco caterpillar, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is one of the economic pest causing serious damages in a large number of cultivating plants worldwide (Bragard et al. 2019) as well as in Indonesia (Ngatimin and Nasruddin 2019), covering 40 plant families (Bragard et al. 2019). It has been reported that *S.*

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litura has a high migratory ability and is widely distributed in both tropical and temperate regions. A report in the Philippines confirmed that *S. litura* caused moderate to severe damages to corn (Gerpacio et al. 2004). The damage caused by this pest on corn in Lampung Province has not been fully revealed.

Recently, the use of insecticides for controlling *S. litura* has continuously increased, making it possible to become resistant. It has been reported that *S. litura* is now being resistant to several groups of insecticides resulting in more difficulties for controlling it. The increase in public awareness on the negative impacts on the use of pesticide, especially on human health and environment has led to finding and developing alternative control strategies eco-friendly. One of which is using entomopathogenic fungi (EPF). Application of EPF has no harmful residues and improves the balance of the bionetwork within the ecosystem. Once the EPF has spread in host populations, it provides lifetimes of pest control (Scheepmaker and Butt 2010).

Exploration to obtain biological control agents, including EPF is the first step in the implementation of the biological control techniques. The EPF can be obtained from the infected insects, soils or plants' rhizosphere (Singh et al. 2016). Tesfaye and Seyoum (2010) reported that temperature conditions were one of the factors that influenced pathogenicity of the isolate of EPF. Since the temperature condition varies among regions, indigenus EPF can be one of the alternatives to provide better results since they are more adapted to the local environment (Sayed et al. 2019).

This study was performed to obtain and identify EPF of *S. Litura,* collected from corn field in 4 corn producing areas in Lampung Province, as well as the potential of EPF against the pest on corn fields in the Province.

Methods

Sampling

Soil samples were collected from corn rhizosphere in 4 regions (districts), namely Bandar Lampung, Pesawaran, Lampung Selatan and Lampung Timur. Three corn fields with a minimum area of 50 m^2 were selected in each region. One corn field was selected in each sub-district. Soil samples were collected from 5 corn plant rhizospheres at 10–15 cm of depth, which were diagonally and randomly chosen. As much as 500 g of soil were taken from each plant's rhizosphere and composited. Totally, 1000 g of the composite soil sample from each field was collected and brought to the laboratory.

Isolation of Entomopathogenic fungi

Isolation of the potential EPF was performed in the Laboratory of Agricultural Biotechnology, Faculty of Agriculture, University of Lampung, Indonesia, using baiting method (Tarasco et al. 2020). The soil samples were sieved by a 600 mesh strainer and moved into plastic trays (35 × 28 × 7 cm). Ten larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) were placed on the soil and covered with filter paper. The trays were then incubated in a dark condition at room temperature. Observation was performed for 14 days on the fungus, which emerged on the body surface of *T. molitor.* The infected *T. molitor* larvae were sterilized by dipping in 1% sodium hypochlorite (NaOCl) for 30 s. The emerging fungus was taken and cultivated on Potato Dextrose Agar (PDA) medium (Himedia, India) and incubated for 7 days at room temperature.

Pathogenicity test of the obtained entomopathogenic fungi

The 3rd instar larvae of *S. litura* were used for pathogenicity tests. Ten larvae were placed into sterile plastic petri dish (9 cm of diameter) containing 7 days old fungi, which were obtained from *T. molitor*. The larvae of *S. litura* were rolled over to make sure that the body surface of the larvae was completely covered by the mycelium or conidia of the fungi. The larvae were then transferred into plastic jars (14 cm of diameter) containing fresh leaves of *Ricinus communis* Linnaeus (Malpighiales: Euphorbiaceae) as food. For the control, the healthy *Spodoptera* larvae were directly placed into a plastic jar. Observation was performed for 14 days on the deaths of *S. litura*. Percentage of mortality (PM) was calculated using formula [(a/b) × 100%]; a = number of the death of *S. litura*; b = Total of *S. litura* observed.

Identification of the entomopathogenic fungi

Identification, conducted to the fungi causing death of *Spodoptera* larvae, was performed based on the sequence analysis of the Internal Transcribed Spacer (ITS) region.

DNA extraction

DNA extraction was conducted based on the method performed by Swibawa et al. (2020). The conidia of 7 days old of *Aspergillus* and 21 days old of *Beauveria* (those were cultured on PDA in sterile plastic petri dish with 9 cm in diameter) were harvested by added with 10 ml of sterile distillate water and carefully grabbed using drigalski. The suspension was then transferred into a 30 ml of centrifuge tube and centrifuged at 14,000 rpm for 10 min, using CF15RXII (Hitachi, Japan). After centrifugation, 1 mL of 70% cold ethanol was directly added into the tube and centrifuged at 14,000 rpm for 10 min. The supernatant was then removed and 1 mL of extraction buffer (0.5 mL Tris HCl, 1 mL SDS 1% + 2.8 mL NaCl, 0.2 mL

Mercaptoethanol, 2 mL EDTA, 3.5 mL sterile water) was added to the tube and suspended. The pellet suspension was shifted into a mortar and incubated at – 40 °C for 24 h. After incubation, the frizzed suspension was pounded until completely crushed. In total, 500 µL of pellet suspension was transferred into a 1.5-mL tube. As much as 400 µL of 2% cetyl trimethylammonium bromide (CTAB) was added, gently homogenized and incubated at 65 °C for 1 h, using a water bath (Brookfield TC 550 MX-230, USA). After incubation, 500 µL of Phenol Chloroform Isoamyl Alcohol (PCI) solution (25: 24: 1) was added, hardly homogenized, and centrifuged at 14,000 rpm for 10 min. In total, 600 µL supernatant was conveyed into a new 1.5-mL tube. As much as 600 µL of Chloroform Isoamyl Alcohol (CI) solution (24:1) was added, homogenized, and centrifuged at 14,000 rpm for 10 min. Totally, 400 µL of the supernatant was relocated into a new 1.5-mL tube. As much as 400 µL cold isopropanol was added into the tube, gently homogenized by hand, and incubated at – 40 °C for 20 min. After incubation, the tube was centrifuged at 14,000 rpm for 15 min. The supernatant was discharged, and 500 µL of cool 70% ethanol was added and centrifuged at 14,000 rpm for 5 min. The supernatant was discharged, and the pellet obtained was air-dried at room temperature for 24 h. After air-dried, 50 µL 1 × Tris–HCL EDTA (TE) pH 8.0 (1st Base Malaysia) was added. All centrifugation processes after incubation at − 40 °C were performed using a centrifuge Microspin12 (Biosan, Latvia).

PCR amplification

Amplification was conducted with Sensoquest Thermal Cycler Machine (Germany). PCR amplification was performed on Internal Transcribed Spacer (ITS) region, using primer ITS1 (5'TCC GTA GGT GAA CCT TGC GG 3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC 3') (White et al. 1990). PCR was performed in total volume 25 µL consist of 12.5 µL Master Mix (Red Mix) (bioline), 1 µL of 10 µM of primer ITS 1 and ITS 4, 1 µL DNA template (~1 µg/µL) and 9.5 µL sterile distilled water. DNA amplification covering one cycle of the initiation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 1 min, annealing at 46–52 °C for 1 min, primer extension at 72 °C for 1 min, and one cycle of elongation at 72 °C for 5 min. The PCR results were electrophoresed by 0.5% agarose gel suspended in 20 mL 1× buffer Tris-Boric Acid-EDTA (TBE) (1st Base Malaysia) containing 1 µL Ethidium Bromide (EtBr 10 mg/mL). The electrophoresis was conducted using a 1× TBE buffer at 50 V for 70 min. The results were visualized using DigiDoc UV transilluminator (UVP, USA).

Sequencing and analysis of the result

The PCR product was sent to 1st Base Malaysia for sequencing. The sequencing results were analyzed using Bio Edit program ver. 7.2.6 for windows and submitted to Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the possible identity. The dendrogram was constructed using Mega 7 program for Windows (Kumar et al. 2016) by neighbor joining method (jukes and cantor model). Reference strains used in this study were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). Detailed information of the reference strains is shown in Additional file 1: Table S1.

Aflatoxin production test

Assessment was performed on the *Aspergillus* spp. showed capability to cause death of *S. litura* based on method described by Fente et al. (2001). The 7 days old isolates of *Aspergillus* spp. were cultivated on yeast extract with supplements (YES) medium (Himedia, India) added to 2% of methyl-ß-cyclodextrin (Sigma Aldrich, USA) and incubated at room temperature. An isolate of *A. flavus* BIO 3338, an aflatoxigenic fungi collection of Indonesian Culture Collection (InaCC), which was isolated from diseased peanuts, was used as positive control. Observation was performed 5 days after incubation under UV light (356 nm). Aflatoxigenic isolates showed fluorescence but not for non-aflatoxigenic isolates (Fente et al. 2001).

Corn damage caused by *Spodoptera litura* in Lampung Province

The damage of corn data was obtained from the survey conducted by the Crop and Horticultural Plant Protection Agency of Lampung Province from 2010 to 2019. Survey performed in all corn producing area in Lampung (Bandar lampung, Lampung Selatan, Lampung Tengah, Lampung Timur, Lampung Utara, Lampung Barat, Metro, Pesawaran, Tulang Bawang, Tulang Bawang Barat, Pesisir Barat and Way Kanan, Pringsewu), using method described by Direktorat Perlindungan Tanaman Pangan (2018). The data collected in this study was the damaged area caused by *S. litura* which were divided into 4 levels namely slight (1 to ≤ 25% of plant damage), medium (> 25 to ≤ 50% of plant damage), severe (> 50 to ≤ 85% of plant damage), and crop failure (> 85% of plant damage).

Weather data in Lampung Province

Rainfall and rainy days were collected from ombrometer collected from 15 sub-districts in Lampung Province, during the years of 2013–2019. Minimum and maximum temperature data were obtained from Statistics of Lampung Province during the year of 2010–2017, which can

be freely downloaded from https://lampung.bps.go.id/subject/151/iklim.html#subjekViewTab3.

Statistical analyses

Pathogenicity test was arranged using Completely Randomized Design (CRD) with 3 replicates. The data was analyzed by one-way analysis of variance (ANOVA). If there is a significant difference between the means of two or more isolates, further analysis was carried out using Least Significant Difference (LSD) test. Statistical analysis was performed with R Statistical Software (version 4.1.1; R Foundation for Statistical Computing, Vienna, Austria).

Results

Fungal isolates obtained from infected *Tenebrio molitor*

Totally, 12 fungal isolates were recovered from the infected *T. molitor* larvae (Additional file 2: Fig. S1) which were previously laid on the 8 soil samples as lure of EPF, *i.e., Kemiling Permai* (1 isolate), *Rajabasa Raya* (1 isolate), *Sukaraja* (2 isolates), *Natar* (2 isolates), *Sidosari* (2 isolates), *Rejoagung* (1 isolate), *Negeri Katon* (2 isolates) and *Balerejo* (1 isolate). The samples were collected from the corn rhizosphere of different varieties, namely sweet corn, NK 22, P27 and an unknown variety representing local varieties planted by the farmers. Eight of the fungal isolates showed white colony color, 3 isolates had green colony color and the other 1 isolate showed black colony color (Table 1).

Pathogenicity of the obtained entomopathogenic fungi to *Spodoptera litura*

Nine out of 12 fungal isolates showed capability to cause mortality of *S. litura*, i.e., NKPT, SKHJ, SDHJ, RAHJ, SDPK and RRPK. Six of the isolates produced mortality less than 50%, namely NTHT (3.33%), BRPT (3.33%), NKPT (3.70%), SDPK (6.67%), RRPK (7.41%) and RAHJ (24.44%) and the other 3 isolates produced more than 50% of mortality, i.e., NKPT (74.07%), SKHJ (60.37%) and SDHJ (50.74%) (Fig. 1). Further identification was conducted on the 4 EPF causing mortality of *S. litura* more than 20%, namely NKPT (74.07%), SKHJ (60.37%), SDHJ (50.74%) and RAHJ (25%) (Fig. 2).

Identity of the entomopathogenic fungi

The result of BLAST searches revealed that NKPT shared 99.62% similarity to *B. bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae) isolate ERL923 (Acc. No. MN122413); meanwhile SKHJ, SDHJ and RAHJ were shared 100% similarity with *Aspergillus aflatoxiformans* Frisvad, Ezekiel, Samson & Houbraken (Eurotiales: Trichocomaceae) and *Aspergillus flavus* Link (Eurotiales: Trichocomaceae) and *Aspergillus oryzae* (Ahlb.) Cohn. (Eurotiales: Trichocomaceae). The result of BLAST was confirmed by the phylogenetic tree. The NKPT isolate was placed within a group of *B. bassiana* ARSEF 7518 (ACC. No. HQ880762), ARSEF 296 (Acc. No. AY532013) and ARSEF 937 (Acc. No. AY532056) (Fig. 3); meanwhile SKHJ, SDHJ and RAHJ were placed in the group of *A. aflatoxiformans, A. flavus* and *A. oryzae* (Fig. 4).

Further investigation to verify the identity of SKHJ, SDHJ and RAHJ isolates was conducted on their capability to produce aflatoxin using YES medium supplemented with 2% of methyl-ß-cyclodextrin described by Fente et al. (2001). The group of *A. flavus* and *A. aflatoxiformans* were reported as producing toxin *Aspergillus* but not for *A. oryzae*. One isolate of producing toxin *A. flavus* BIO 3338 was also included. The result showed that the *A. flavus* BIO 3338 showed green

Table 1 Fungal isolates obtained from infected *Tenebrio molitor*

Regions		Ordinate	Corn varieties	Plant age (days after planting)	Name of the isolate	Colony color on PDA medium
Districts	Sub-districts					
Bandar Lampung	Kemiling Permai	− 5.3822191, 105.2201871	Sweet corn	10	KPPK	White
	Rajabasa Raya	− 5.348984, 105.244406		50	RRPK	
Lampung Selatan	Sukaraja	− 5.685002, 105.673718	Sweet corn	42	SKPK	
					SKHJ	Green
	Natar	− 5.343718, 105.230879	NK22	60	NTPK	White
					NTHT	Black
	Sidosari	− 5.322588, 105.250467	Sweet corn	60	SDPK	White
					SDHJ	Green
Pesawaran	Rejoagung	− 5.183594, 105.200795	P27	15	RAHJ	
	Negeri Katon	− 5.311441, 105.1041564		30	NKPT	White
					NKPK	
Lampung Timur	Balerejo	− 5.1094025, 105.3831026	Unknown (local variety)	60	BRPT	

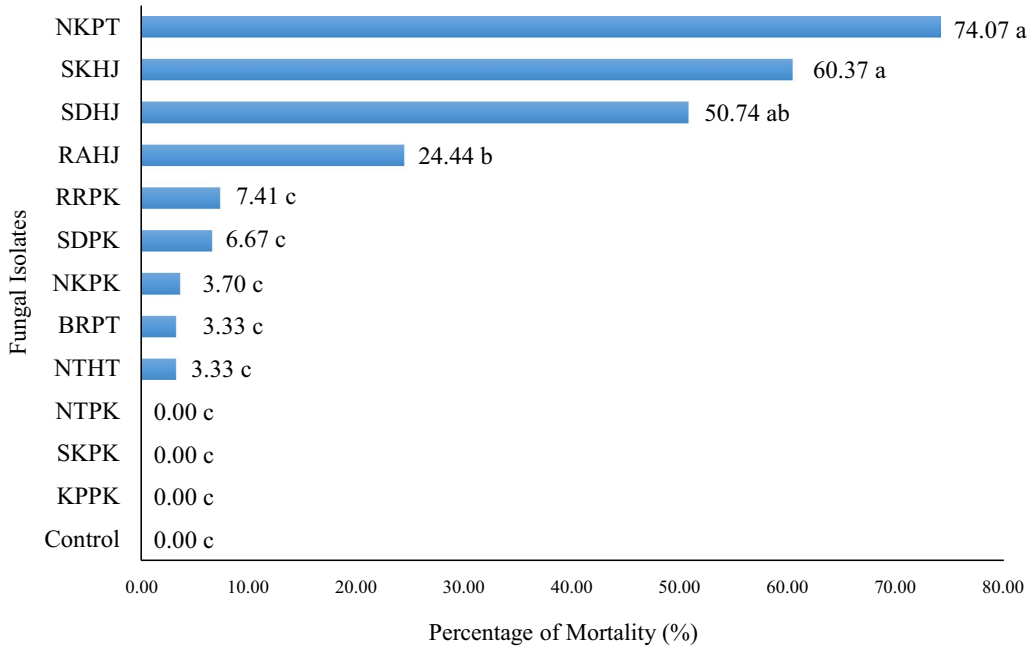

Fig. 1 Percentage of mortality of *Spodoptera litura* caused by fungal application. The numbers followed by the same letter are not significantly different based on least significant difference test (LSD) analysis at the 5% of significance level. Statistical analysis was performed on the data which were transformed using $\sqrt{x}+0.5$. Treatments were arranged using a completely randomized design (CRD). The data are means collected from observation of the mortality of 10 individuals of *S. litura* applied with entomopathogenic fungi. Assessment was conducted in 3 replicates

fluorescence but not for SKHJ, SDHJ and RAHJ (Fig. 5). Inability SKHJ, SDHJ and RAHJ produced green fluorescence indicated that the three isolates were not able to produce aflatoxin, confirming that SKHJ, SDHJ and RAHJ were *A. oryzae.*

Corn damage caused by *S. litura* in Lampung Province

The invasion of *S. litura* mostly caused slight damage on corn (1 to $\leq$25% of plant damage). The damaged area generally decreased, from 717 ha in 2010 to 169 ha in 2019. The lowest damaged area was found in 2015, which affected 10 ha with slight plant damage with no record of medium damage, severe damage and crop failure. Medium plant damage occurred in 2011, 2012, 2018 and 2019 affected 29, 2, 3 and 1 ha of corn fields, respectively. Severe plant damage was observed in 2011, affected 25 ha of corn fields. Crop failure conditions were faced in the year 2018 affected 39 ha of corn fields (Fig. 6). The damage caused by *S. litura* was initially observed in January and continued rising until July. The damage decreased in August and September and again raised in October to December, with the most damage observed in November. During a year observation, the lowest damage was found in August and September (Fig. 7).

Weather conditions in Lampung Province

In January, heavy rainfall occurred (274.70 mm) and then decreased in the next months. During July to September, rainfall was lower than 100 mm, with the lowest rainfall observed in August (54.88 mm). The rainfall increased above 100 mm in October (103.64 mm) and increased until December (256.14 mm). The highest rainy days were observed in January (19.39 days) and continue to decrease in the months after. The smallest rainy days occurred in August (2.52 days) and September (2.83 days). The amount of rain increased in October (4.05 days) and continued until December (13.89 days). The minimum temperatures were 22.15–23.66 °C; meanwhile the maximum temperature was in the range of 31.30–37.54 °C. The highest temperature occurred in September (37.54 °C).

Discussion

Twelve isolates of EPF were recovered from 8 soil samples collected from the districts of Bandar Lampung, Lampung Selatan, Pesawaran and Lampung Timur. The fungi were obtained by a baiting method employing *T. molitor*. Baiting method using *T. molitor* (Sharma et al. 2018) has been reported as one of methods used to

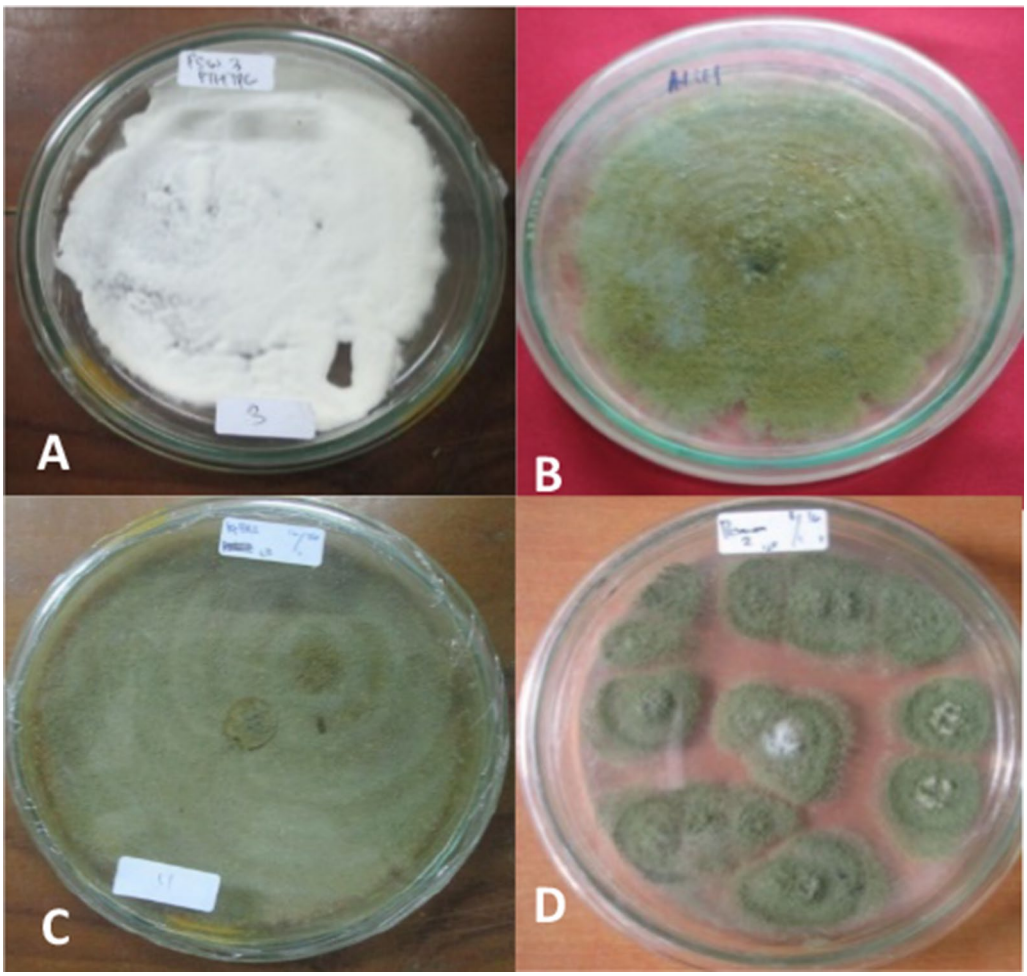

Fig. 2 Prospective fungal isolates causing death of *Spodoptera litura* more than 20%. **a** NKPT, **b** SKHJ, **c** SDHJ, **d** RAHJ

obtain EPF from soil such as *B. bassiana* (Sharma et al. 2018).

Six out of 12 fungal isolates obtained in this study were confirmed causing mortality to *S. litura* (3.57–75%). The highest mortality rate was produced by NKPT (75%), followed by SKHJ (60.71%), SDHJ (50%), RAHJ (25%), SDPK (3.57%) and RRPK (3.57%). Some natures of EPF influenced their capability to cause death of herbivorous insects, *i.e.,* spore production and viability (Rosmiati et al. 2018), penetration (Mora et al. 2017), infection (Santos et al. 2018) and enzymes produced by each EPF (Dhawan and Joshi 2017). Different EPF produce several enzymes and toxins. Chitinase was produced by *Aspergillus* spp., which can degrade chitin of fungal pathogens and pest insects (Purkan and Sayyidah 2016).

Further identification was performed on the 4 isolates which produced mortality more than 20%, *i.e.,* NKPT, SKHJ, SDHJ and RAHJ. Based on the BLAST search result, the NKPT was closely related with the group of *B. bassiana*; meanwhile SJHJ, SDHJ and RAHJ shared 100% similarity with *A. aflatoxiformans*, *A. flavus* and *A. oryzae*. Based on the phylogenetic tree analysis revealed that NKPT was placed within the group of *B. bassiana* (Fig. 3); meanwhile, SJHJ, SDHJ and RAHJ were placed within a group of *A. aflatoxiformans* and *A. flavus* and *A. oryzae* (Fig. 4). In the case of NKPT, the isolate was *B. bassiana*; meanwhile the other 3 isolates should be carefully analyzed on their characters to define their species identity.

The genus of *Aspergillus* was divided into 4 subgenera (Aspergillus, Circumdati, Fumigati and Nidulantes) and 20 sections. *A. oryzae* is a representing domesticated group of *A. flavus*. This species can be differentiated from the other members of *A. flavus*-clade on their inability to produce aflatoxin (Frisvad et al. 2019).

The three isolates of *Aspergillus* found in this study (SKHJ, SDHJ and RAHJ) were confirmed on their inability to produce aflatoxin. They did not produce

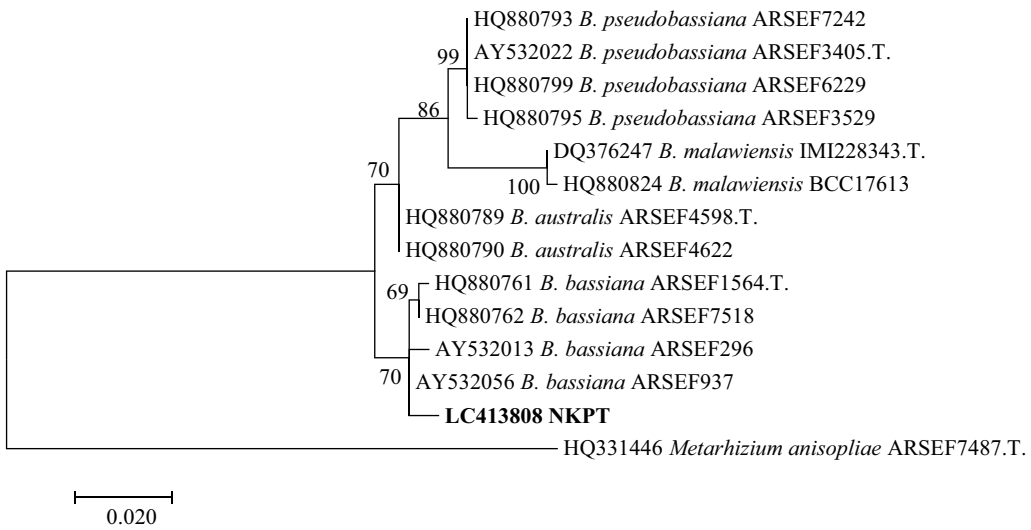

Fig. 3 Phylogenetic tree developed based on sequence analysis of ITS region by primer ITS1 and ITS4 with Maximum Likelihood method (Tamura–Nei model) created using MEGA7 for windows (Kumar et al. 2016). NKPT isolate placed within group of ex type and reference strains of *Beauveria bassiana*, i.e., ARSEF3405.T. (Acc. No. AY532022), ARSEF937 (Acc. No. AY532056), isolate ARSEF296 (Acc. no. AY532013) and ARSEF7518 (Acc. No. HQ880762). NKPT has other designations as B1_UNILA. *Metarhizium anisopliae* ARSEF7487.T. (Acc No. HQ331446) was used as out group. Reference strains included in this study were assembled from the study of Rehner and Buckely (2005), Rehner et al. (2006) and Rehner et al. (2011). T = ex type isolate

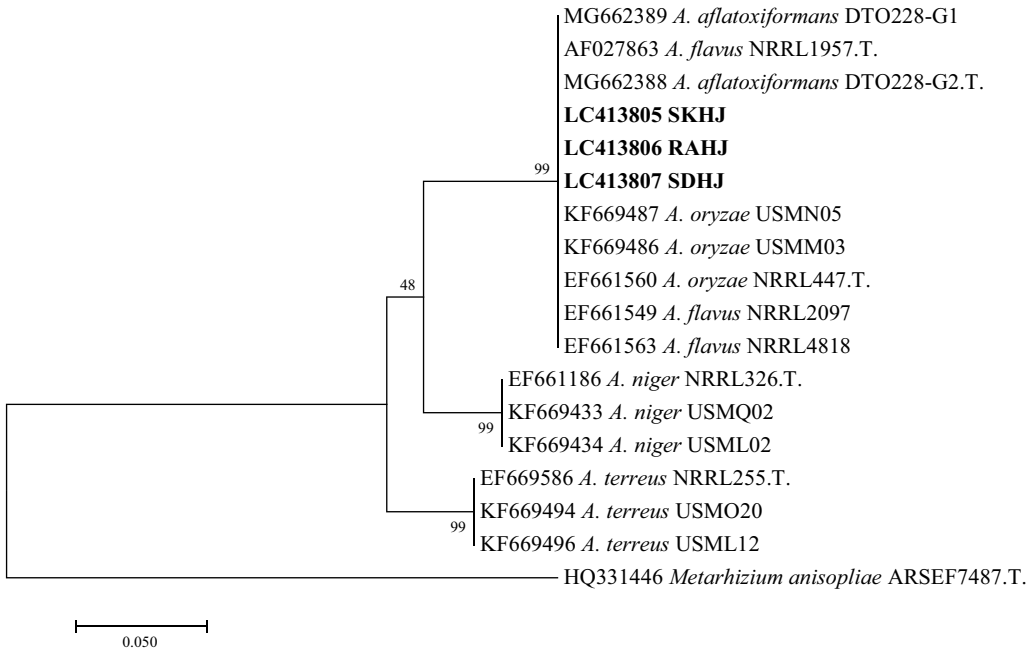

Fig. 4 Phylogenetic tree developed based on sequence analysis of ITS region by primer ITS1 and ITS4 with Maximum Likelihood method (Tamura–Nei model) created using MEGA7 for windows (Kumar et al. 2016). Isolate of SKHJ, SDHJ and RAHJ placed within group of ex type isolate and reference strains of *Aspergillus oryzae* (NRRL447.T., Acc. EF661560; USMN03, Acc. No. KF669486; USMN05, Acc. No. KF669487), *A. flavus* (NRRL1957.T., Acc. No. AF027863; NRRL2097, Acc. No. EF661549; NRRL4818, Acc. No. EF661563) and *A. aflatoxiformans* (DTO228-G2.T., Acc. No. MG662388; DTO228-G1, Acc. No. MG662389). SKHJ has other designations as AP6_UNILA, SDHJ has other designations as AP9_UNILA, RAHJ has other designations as AP7_UNILA. *Metarhizium anisopliae* ARSEF7487.T. (Acc No. HQ331446) was used as out group. Reference strains included in this study were assembled from the study of Peterson (2008), Schneider et al. (2011), Zulkifli and Zakaria (2017) and Frisvad et al. (2019). T = ex type isolate

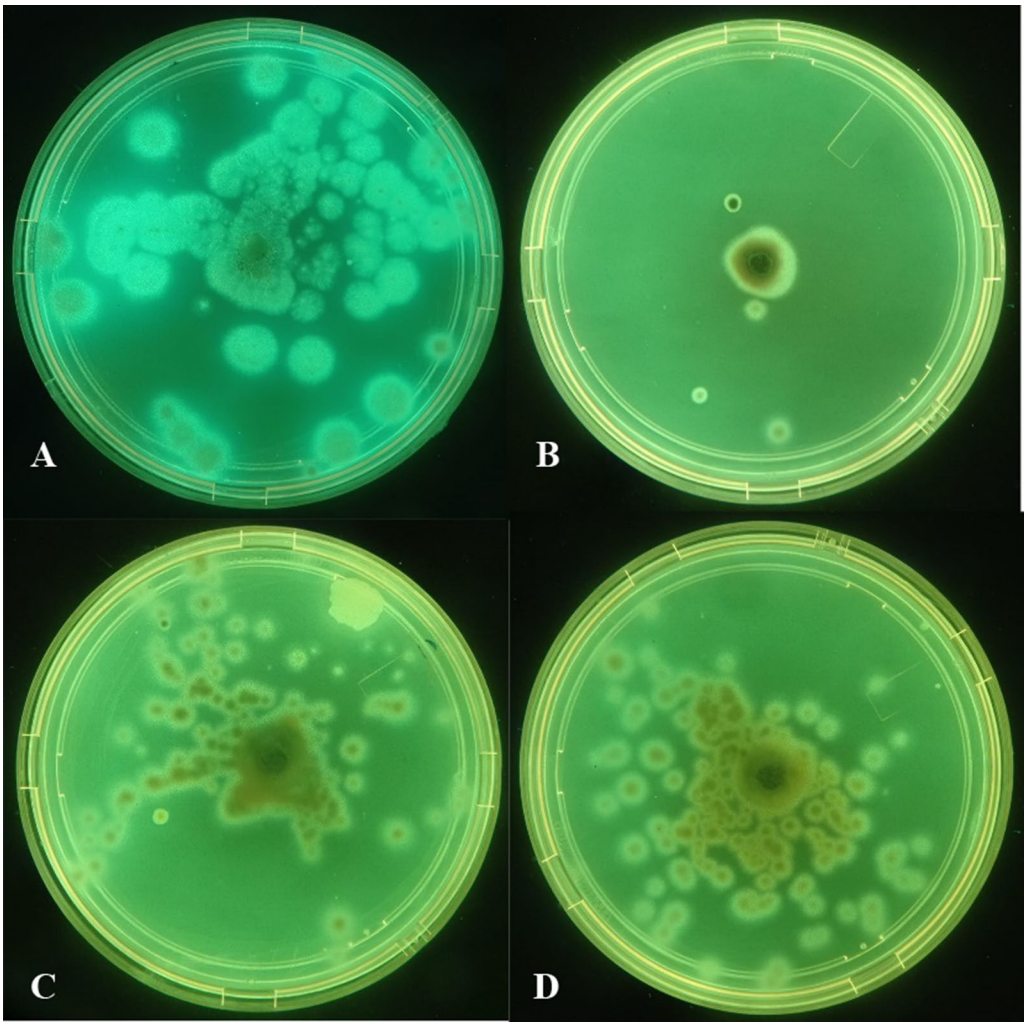

Fig. 5 Fluorescence of 5 days old *Aspergillus* isolate grown on YES medium supplemented with 2% of methylated ß-cyd observed under UV light (365 nm). **a** *A. flavus* BIO 3338, **b** SKHJ, **c** RAHJ, **d** SDHJ

any fluorescence when they were cultured on the YES medium supplemented with 2% of methyl-ß-cyclodextrin. The fluorescence was observed on *A. flavus* BIO 3338, an aflatoxin producing-isolate, which was used as a positive control. YES medium, which was added by 2% of methyl-ß-cyclodextrin, was reported to be used to differentiate *Aspergillus* aflatoxin producing-isolate from non-aflatoxin producing-isolate by its capability to produce fluorescence when it was observed under UV light (Fente et al. 2001). Here, it was concluded that SKHJ, SDHJ and RAHJ isolates were *A. oryzae*. To our knowledge, this is the first report of *A. oryzae* isolates as an EPF of *S. litura* in Indonesia.

Aspergillus oryzae was established and initially used for food production about 2000 years ago in China (Baker and Bennett 2008). As well as in China, this fungus is also extensively employed for food production in Japan, Korea, Thailand and Indonesia (Baker and Bennett 2008). The beneficial aspect of *A. oryzae* other than its use in food production, especially as a biological control agent, has not been widely reported. The role of *A. oryzae* as EPF was firstly reported in 2015 (Zhang et al. 2015). They described an isolate of *A. oryzae,* namely XJ-1, showed capability as EPF on locusts. In this study, the 3 isolates of *A. oryzae* (SKHJ, SDHJ and RAHJ) were found pathogenic to *S. litura*. Since *A. oryzae* is not pathogenic to humans, animals or plants (Chuang et al. 2019), it has Generally Regarded as Safe (GRAS) status from Food and Drug Administration (FDA) United States of America (Sewalt et al. 2016). This *A. oryzae* has a great potential to be developed for biological control agent of herbivorous insects.

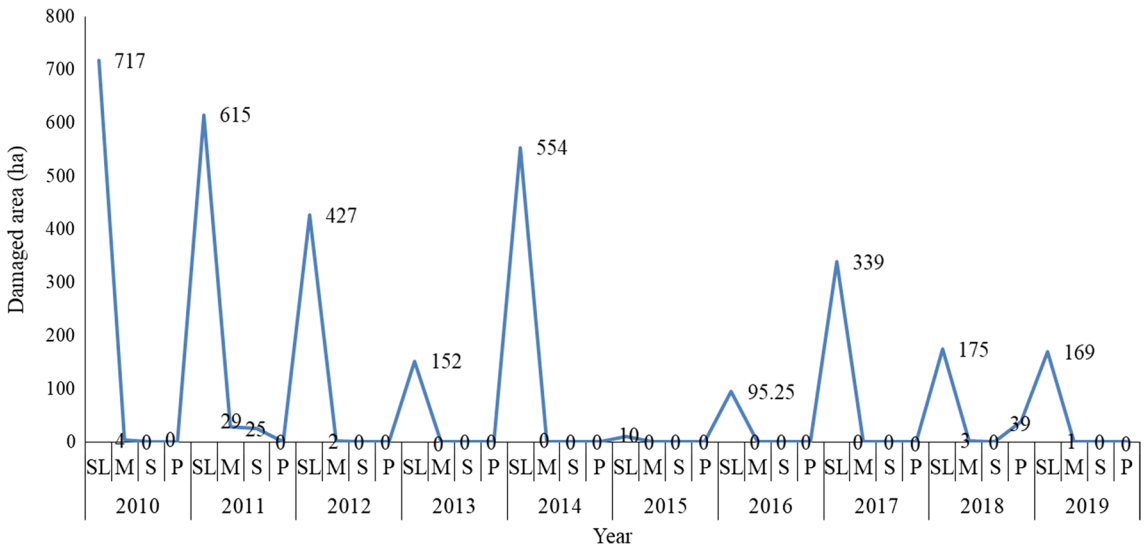

Fig. 6 Damaged area of corn field caused by *Spodoptera litura* in Lampung during 2010–2019. SL: slightly damage (1 to ≤ 25% of plant damage), M: medium damage (> 25 to ≤ 50% of plant damage), S: severe damage (> 50 to ≤ 85% of plant damage), P: crop failure (> 85% of plant damage)

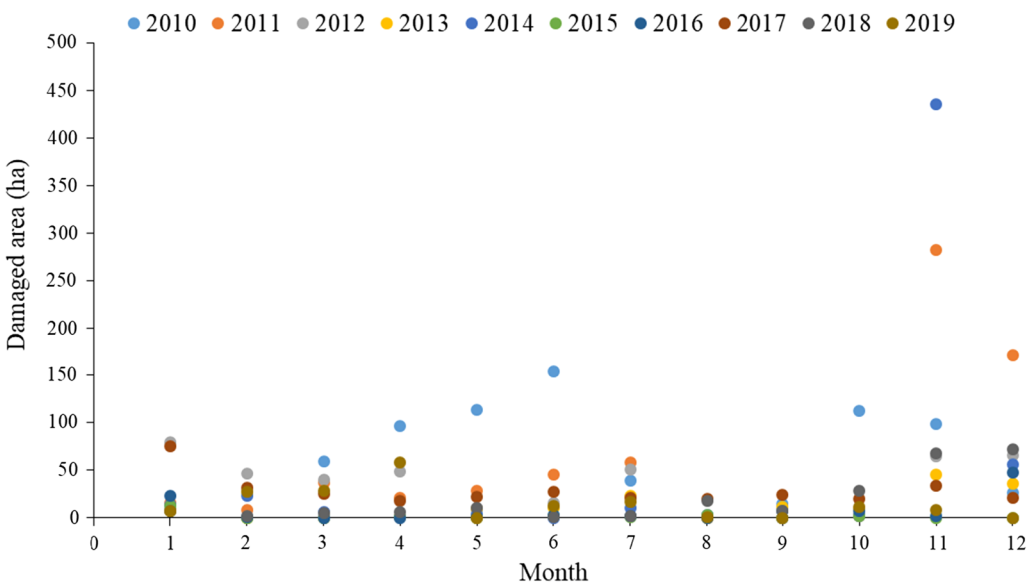

Fig. 7 Damaged area of corn field caused by *Spodoptera litura* in Lampung each month during 2010–2019

In this study, native corn isolates of EPF belong to *B. bassiana* (NKPT) and *A. oryzae* (SKHJ, SDHJ and RAHJ) as pathogen of *S. litura* infested corn were confirmed. Pathogenicity tests revealed the potential of EPF to be used as a biological control agent against target pest. The EPF found in this study (NKPT, SKHJ, SDHJ and RAHJ) originated from the corn rhizosphere. Here, it will provide more advantages if they were used for controlling *S. litura* on corn, since it is already well adapted to the corn field environment. However, further study is needed, especially on their optimum capability for controlling *S. litura* in the field as well as their stability and tolerance to environmental pressure and their effect on non-target organisms including natural enemies.

Application of EPF has been reported to have no or limited adverse effect on some natural enemies (Roy and Pell 2000). For example, application of *B. bassiana* showed no negative effect on the survival, duration, adult

longevity, and fecundity of predator *Coccinella undecimpunctata* L. and *Hippodamia variegate* L. (Sayed et al. 2021). The EPF, *Metarhizium brunneum*, could be potentially applied against *Delia radicum* with a limited danger to its parasitoid *Trybliographa rapae* (Rännbäck et al. 2015). On the other hand, some other natural enemies have been reported negatively influenced by EPF application (Abbas 2020). Both strains of *B. bassiana* (AL1 and ATCC 74040) have also been reported negatively affecting *Encarsia formosa*, a parasitoid of *Trialeurodes vaporariorum* (Oreste et al. 2016).

Fluctuate damage of corn caused by *S. litura* was recognized in Lampung Province. The damage was slight (1 to ≤ 25% of plant damage) damage to crop failure (> 85% of plant damage) with slight damage being the most damage observed in each year. The medium (> 25 to ≤ 50% of plant damage) and severe (> 50 to ≤ 85% of plant damage) damage were rarely found in the field. During 2010–2019, the lowest damage area was observed in 2015, with 10 ha of slight plant damage without any medium damage, severe damage and crop failure.

Research performed by Fand et al. (2015) revealed that the development of all the immature stages of *S. litura* linearly increased until on or about 34–36 °C, but not after this range of temperature. The temperature of 38 °C caused lethal to larval and pupal stages of *S. litura* and there will be no development to the next stage. Females were unable to lay eggs at low (15 °C) and high (> 35 °C) temperatures. The highest damage of *S. litura* on cotton was observed when maximum temperature was in the range of 32–35 °C and the minimum temperature was 24–26 °C (Selvaraj et al. 2010). Alteration of temperature negatively influenced feeding performance of 2nd instar of *S. litura* to yellow cress (*Rorippa dubia*) (Pham and Hwang 2020). The outbreaks of this pest insect also occurred in heavy rainfall conditions after a long dry spell (Thanki et al. 2003).

The lowest damage of corn in Lampung Province was found in August and September gets together with high temperature and low rainfall.

Conclusions

Two species groups of EPF, *i.e., Aspergillus oryzae* and *Beauveria bassiana* were confirmed to be potentially used to control *S. litura* infesting corn in Lampung Province, Indonesia. It seems that this is the first report of the isolates of *A. oryzae* as EPF of *S. litura* in Indonesia. Identity of *A. oryzae* was verified by its inability to produce aflatoxin. Two isolates of *A. oryzae* (SKHJ and SDHJ) showed pathogenicity which was not significantly different from *B. bassiana* (NKPT). On the other hand, one of the isolates of *A. oryzae* (RAHJ) displayed significantly lower pathogenicity than *B. bassiana*. This isolate (RAHJ), however, showed pathogenicity which was not significantly different from the two other isolates of *A. oryzae* (SKHJ and SDHJ).

Abbreviations

A. Aflatoxiformans: *Aspergillus Aflatoxiformans*; *A. flavus*: *Aspergillus flavus*; *A. oryzae*: *Aspergillus oryzae*; *B. bassiana*: *Beauveria bassiana*; BLAST: Basic Local Alignment Search Tool; CRD: Completely randomized design; CI: Chloroform Isoamyl Alcohol; CTAB: Cetyl trimethylammonium bromide; EDTA: Ethylenediaminetetraacetic acid; InaCC: Indonesian Culture Collection; ITS: Internal Transcribed spacer; LSD: Least significance different; *M. robertsii*: *Metarhizium robertsii*; PCI: Phenol Chloroform Isoamyl Alcohol; PM: Percentage of Mortality; SDS: Sodium Dodecyl Sulfate; *S. litura*: *Spodoptera litura*; TBE: Tris HCL Boric Acid EDTA; *T. molitor*: *Tenebrio molitor*; YES: Yeast extract with supplements.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-021-00473-8.

Additional file 1. Reference strains used in this study.

Additional file 2. Infected *Tenebrio molitor* cadaver at 14 days after laid on each of soil samples.

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Authors' contributions

YF and RS considered and planned the experiment. BS, II and SS carried out the isolation and pathogenicity test including rearing of *Spodoptera litura* for pathogenicity test. LTP, YF and RS performed molecular work and analysis. MH and RAR collected data on the plant damage area caused by *S. litura* as well as weather data. IGS and PP performed analysis and interpreted the plant damage and weather data. YF and RS prepared the manuscript. The authors provided responses and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and material

The datasets used or analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest regarding the publication of this manuscript.

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