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Isolation and screening of entomopathogenic fungi against the grasshopper *Oxya chinensis* (Orthoptera: Acrididae) from rice fields in Thua Thien Hue, Vietnam

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Abstract. *Cuong LCV, Souvannalath B, Ha TP, Lien LQ, Tam VTT, Hoai PTT, Anh HLT, Dat TTH. 2022. Isolation and screening of entomopathogenic fungi against the grasshopper* Oxya chinensis *(Orthoptera: Acrididae) from rice fields in Thua Thien Hue, Vietnam. Biodiversitas 23: 4906-4911.* The grasshopper *Oxya chinensis* is one of the most abundant herbivorous insects of rice plants in Vietnam and causes severe damage to the crop. Therefore, biological control has become a particularly valuable method for grasshopper and insect control in agricultural production. The aim of the present study was to isolate and investigate the potential entomopathogenic fungi against the grasshopper *O. chinensis* from rice fields in Thua Thien Hue, to develop environmentally friendly bio-insecticides. The results showed that 37 fungal isolates were isolated from dead grasshoppers collected from rice fields in Thua Thien Hue province. Results of pathogenicity screening of 12 selected fungal isolates showed that the mortality rate of 4th-instar grasshopper nymphs *O. chinensis* ranged from 21.99% to 100% after 15 treatment days at a concentration of 1×10^8 conidia/ml. Of these, three fungal isolates, namely NL3, NL4, NL12 showed the highest pathogenicity against the grasshopper *O. chinensis* with mortality rates of 87.73%, 89.81%, and 100%, respectively after 15 days. LC_{50} values of NL3, NL4, NL12 isolates against 4th-instar nymphs and adults of *O. chinensis* ranged from 9.5×10^4 to 4.8×10^5 conidia/mL and 7.8×10^6 to 2.5×10^6 conidia/mL, respectively at 7 days. LT_{50} values of NL3, NL4, NL12 isolates against 4th-instar nymphs and adults of *O. chinensis* were from 4.1 to 4.9 days and 5.5 to 6.1 days, respectively at a concentration of 1×10^8 conidia/mL. Results from morphological identification and nuclear ribosomal internal transcribed spacer (ITS) gene sequence showed that three potential entomopathogenic fungi were *Aspergillus tamarii*, *Beauveria bassiana*, and *Metarhizium anisopliae*. The pathogenicity of *Aspergillus tamarii*, *Beauveria bassiana*, and *Metarhizium anisopliae* against the grasshopper *O. chinensis* is reported for the first time. The present investigation revealed that these entomopathogenic fungi could be a useful source for developing environment-friendly bio-insecticides for sustainable agricultural production.

Keywords: *Aspergillus tamarii*, *Beauveria bassiana*, entomopathogenic fungi, grasshopper, *Metarhizium anisopliae*, *Oxya chinensis*

INTRODUCTION

Vietnam is one of the countries with developed agriculture and significant contributions to the country's economic development. The main agricultural products of Vietnam include rice, coffee, pepper and cashew nut. Of these, rice production is the most important of Vietnam's agricultural production and one of the world's leading exporters of rice (JICA 2013; Maitah et al. 2020). However, Vietnam's agricultural production is facing many challenges, especially plant pathogenic pests and diseases. Therefore, the use of chemical pesticides is one of the inevitable solutions in agricultural production. Unfortunately, the abuse and excessive use of chemical pesticides in agricultural production has greatly affected the ecological environment and human health (Nguyen et al. 2009; Nguyen 2017). So, there is an urgent need to use biological pesticides to develop agriculture sustainability and ensure the safety of the ecological environment and human health.

Grasshoppers and locusts are among the pests of rice. Rice grasshoppers feed on leaf tissue, big pieces of leaf blades and even panicles. Nymphs eat newly germinated rice seedlings and nurseries, causing them to wither, whereas adult grasshoppers feed on the leaves, shoots, and the bases of maturing earheads, causing them to dry up (Karim and Riazuddin 1999; Shepard et al. 1995). Among rice grasshoppers, *Oxya chinensis* is the most widely distributed species in Asia and one of the major pests of rice, sugar cane, maize, sorghum, beans, wheat, cotton, and reeds. It feeds the leaves of young seedlings and causes considerable damage to rice production (Huadi et al. 2007). In Vietnam, the common rice grasshoppers are *Oxya* spp., including *O. agavisa*, *O. chinensis*, *O. hyla*, *O. japonica*, *O. velox*, *O. rufipes* (Kim and Pham 2014).

Entomopathogenic fungi are a group of fungi that can infect and kill insects and arthropods. Currently, entomopathogenic fungi are classified into the divisions Ascomycota, Zygomycota, Deuteromycota, Oomycota, Chytridiomycota (Araújo and Hughes 2016). To date, over

700 species from approximately 90 different genera have been identified as entomopathogenic fungi and many commercial mycoinsecticides are being developed (Khachatourians and Qazi 2008); however most commercial products are based on several genera or species, such as *Beauveria bassiana*, *Metharhizum anisopliae* and *Isaria fumosoroseus* (Bamisile et al. 2021; Rajula et al. 2021). As a type of ecological pesticide, entomopathogenic fungi are currently used worldwide for the control of a wide range of arthropod pests, particularly pests in agricultural production due to environmental and food safety concerns (Bamisile et al. 2021; Rajula et al. 2021).

Previous studies have shown that *Beauveria*, *Metharhizum*, and *Aspergillus* are potential entomopathogenic fungi to control various grasshoppers and locusts. *M. anisopliae* has been reported to show pathogenicity against the grasshoppers *Poekilocerus pictus*, *Uvarovistia zebra*, *Melanoplus bivittatus*, and locust *Locusta migratoria* (Adatia et al. 2010; Mohammadbeigi and Port 2015; Jiang et al. 2019; Ramanujam et al. 2021), whereas *B. bassiana* has also been reported to show pathogenicity against the grasshoppers *P. pictus*, *U. zebra*¸ *Angaracris* sp. (Otgonjargal et al. 2014; Mohammadbeigi and Port 2015; Ramanujam et al. 2021). Also, *Aspergillus* spp. has been reported as potential entomopathogenic fungi for the control of various grasshoppers and locusts. *A. oryzae* showed pathogenicity against the locust *L. migratoria*, whereas *A. flavus*, *A. fumigutus*, and *A.niger* showed pathogenicity against grasshoppers *Oxya velox*, *P. pictus*, and *Hieroglyphus nigrorepletus* (Zhang et al. 2015; Baskar et al. 2020). However, there has been little research on entomopathogenic fungi against the grasshopper *O. chinensis* so far. The aim of the present study was to isolate and screening of entomopathogenic fungi against the grasshopper *O. chinensis* from rice fields in Thua Thien Hue, Vietnam, in laboratory conditions to develop environmentally friendly bio-insecticides for agricultural production in general and rice production in particular.

MATERIALS AND METHODS

Collection of grasshopper samples

The grasshopper samples (*Oxya chinensis*) were collected from the rice fields in Huong Tra district, Thua Thien Hue province. The samples were stored in sterilized falcon tubes and immediately transferred to the laboratory and isolated fungi.

Isolation of fungi from dead grasshopper samples

The fungi were isolated directly from dead grasshopper samples by transferring external conidia onto PDA plates (Potato Dextrose Agar, Himedia, India) supplemented with 100 µg/mL chloramphenicol and 50 µg/mL streptomycin sulfate to prevent bacterial growth. The plates were incubated at 25 ± 2°C for 1-2 weeks. Fungal colonies with different morphology were transferred onto fresh PDA plates until pure cultures were obtained. The pure cultures were stored in glycerol 25% at -80°C. Colony morphology of fungal isolates was observed on pure cultures of PDA plates, while microscopic characteristics of fungal isolates were observed under the Microscope BX43 (Olympus, Japan) at 40X magnification.

Screening of entomopathogenic fungal isolates against the grasshopper *Oxya chinensis*

To prepare a conidial suspension, fungal isolates were grown on PDA plates for 2 weeks at 25 ± 2°C, and then conidia were harvested and suspended in sterile 0.02% Tween 20. The conidial suspension was vortexed for 2 min to homogenate the suspension and then the concentration of suspension was adjusted to 1×10^8 conidia/mL with a hemocytometer.

For the screening of entomopathogenic fungi against the grasshopper *O. chinensis*, twenty 4th-instar nymphs were dipped in conidia suspension (1×10^8 conidia/mL) for 2-3 seconds to contact with conidia. The sterile 0.02% Tween 20 aqueous solution was used as a control. Then, grasshoppers were transferred into a plastic box and kept for 15 days at 25 ± 2°C under conditions of 75 ± 5 % relative humidity and 12L:12D photoperiod. Several small pieces of rice leaves were provided as food. The mycelial growth of fungi and the number of dead grasshoppers were checked after 5, 7, 9, 12, and 15 days.

The percentage of mortality in grasshoppers was calculated using the following formula of Abbott's (Abbott 1925):

$$P\ (\%) = [C - T/C] \times 100$$

Where: P = estimated percentage of grasshoppers killed by fungal isolates, C = percentage of control living grasshoppers, and T = percentage of treated grasshoppers that survived after the experimentation period.

Determination of lethal concentration and lethal time

Fungal isolates with the highest pathogenicity were determined by their lethal concentrations (LC_{50}) and lethal times (LT_{50}) against 4th-instar nymphs and adults of *O. chinensis*. The LC_{50} values were calculated with different concentrations from 1×10^4 to 1×10^8 conidia/mL and mortality was counted after 7 days. The LT_{50} values were calculated using a concentration of 1×10^8 conidia/mL at different times from 2 to 8 days of treatment.

Identification of entomopathogenic fungi by ITS gene sequences

Genomic DNA of fungal isolates was extracted using QIAamp® DNA Mini Kit according to the manufacturer's instructions. The nuclear ribosomal internal transcribed spacer (ITS) region of fungal isolates was amplified with primers ITS4 and ITS5 in 25 µL PCR reactions (White et al. 1990). PCR reaction (25 µL) included 2.5 µL of 10X standard Taq buffer, 0.5 µL of 10 mM dNTPs, 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 0.5 µL of DNA template, 0.125 µL of *Taq* DNA polymerase, and 20.375 µL of ddH_2O. The steps of reaction were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of DNA denaturation at 94°C for 45 s, primer annealing at 55°C for 45 s, and DNA synthesis extension at

72°C for 45 s, followed by a single step of final extension at 72°C for 10 min. The PCR products were checked on 1.5% agarose gel. The PCR products were sequenced on sequencer ABI PRISM 3100. The obtained sequencing data was assessed their quality by examining their chromatograms, and then trimmed low-quality base calls from the beginning and the end of sequences using BioEdit software v.2.7.5 to obtain high-quality sequences. The high-quality ITS sequences were compared to sequences in the GenBank database (NCBI) to find their highest similarity sequences using the BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and then sequences were aligned using the ClustalW algorithm. The phylogenetic tree of ITS sequences was created by the Neighbor-Joining algorithm with 1000 bootstraps using MEGA software v.7.0.0.

Data analysis

Experiments were performed by completely randomized design and statistical data were calculated using IRRISTAT software v.5.0 with p-value < 0.05. The lethal concentration (LC_{50}) values and lethal time (LT_{50}) values were calculated according to Finney's Probit analysis using SPSS software v.20.0.

RESULTS AND DISCUSSION

Isolation and screening of entomopathogenic fungal isolates against the grasshoppers (*O. chinensis*)

From the dead grasshopper samples collected from the rice fields in Thua Thien Hue, 37 fungal isolates were isolated on PDA plates. Among them, 12 fungal isolates with different colony morphology were selected for the screening of their pathogenicity against the grasshopper *O. chinensis*. The pathogenicity screening of fungal isolates against 4th-instar grasshopper nymphs of *O. chinensis* is presented in Table 1.

The pathogenicity screening results showed that mortality rates of 4th-instar grasshopper nymphs of *O. chinensis* ranged from 21.99 to 100 % after 15 treatment days at a concentration of 1×10^8 conidia/mL. Of these, three fungal isolates, namely NL3, NL4, and NL12 showed the highest pathogenicities against the grasshopper *O. chinensis* with mortality rates of 87.73%, 89.81%, and 100%, respectively after 15 treatment days.

Determination of lethal concentration and lethal time

Three fungal isolates (NL3, NL4, NL12) with the highest pathogenic activities were evaluated for their lethal concentrations (LC_{50}) and lethal times (LT_{50}) against 4th-instar nymphs and adults of *O. chinensis*. Bioassay results revealed that LC_{50} values of NL3, NL4, NL12 isolates against 4th-instar nymphs of *O. chinensis* were 9.5×10^4, 1.6×10^5, and 4.8×10^5 conidia/mL, respectively, and against adults of *O. chinensis* were 7.8×10^6, 1.5×10^6, and 2.5×10^6 conidia/mL, respectively after 7 treatment days (Table 2).

LT_{50} values of three fungal isolates (NL3, NL4, NL12) against 4th-instar nymphs of *O. chinensis* were 4.1, 4.4, and 4.9 days, respectively, while against the adults of *O. chinensis* were 5.5, 5.9, and 6.1 days, respectively at a concentration of 1×10^8 conidia/mL (Table 3). According to the LC_{50} and LT_{50} values, the efficacy of three fungal isolates was in the order: NL12 > NL4 > NL3.

Macroscopic and microscopic characteristics of entomopathogenic fungal isolates

Three fungal isolates (NL3, NL4, NL12) with the highest pathogenic activities against the grasshopper *O. chinensis* were identified based on macroscopic and microscopic characteristics (Figure 1).

Colonies of isolate NL3 were 32-39 mm in diameter after 7 days of incubation at 25°C on PDA. The colonies were cinnamon in color with white mycelia and rough conidia. Based on microscopic observation, conidial heads were biseriate and radiated with the globose vesicle. The conidiophore stipe had rough and uncolored walls. Conidia were globose, and 3.0-5.0 µm. These macroscopic and microscopic characteristics of the fungal isolate NL3 were similar to the typical macroscopic and microscopic characteristics of the genus *Aspergillus*.

Table 1. The mortality rate of 4th-instar grasshopper nymphs of *Oxya chinensis* after treatment with the fungal isolates

Fungal isolates	Mortality rate (%)				
	5 days	7 days	9 days	12 days	15 days
NL1	3.33 ± 2.89^{a}	5.09 ± 0.15^{a}	15.82 ± 0.83^{a}	21.62 ± 3.71^{ab}	23.84 ± 4.62^{ab}
NL2	0	6.75 ± 2.81^{a}	12.30 ± 3.07^{a}	17.57 ± 5.57^{ab}	27.78 ± 4.81^{ab}
NL3	26.67 ± 5.77^{b}	40.61 ± 4.11^{b}	57.73 ± 6.46^{c}	70.75 ± 4.17^{d}	87.73 ± 6.60^{e}
NL4	31.67 ± 2.98^{c}	42.28 ± 6.87^{b}	61.15 ± 7.75^{c}	72.49 ± 6.89^{d}	89.81 ± 7.22^{e}
NL5	0	8.51 ± 3.05^{b}	17.49 ± 2.22^{a}	19.42 ± 6.03^{ab}	23.84 ± 4.62^{ab}
NL6	0	6.75 ± 2.81^{a}	13.97 ± 2.50^{a}	15.60 ± 2.73^{a}	21.99 ± 3.13^{a}
NL7	0	0	12.30 ± 3.07^{a}	21.50 ± 2.47^{ab}	27.78 ± 4.81^{ab}
NL8	0	0	15.72 ± 5.01^{a}	35.38 ± 2.08^{c}	41.90 ± 3.82^{cd}
NL9	0	5.09 ± 0.15^{a}	13.97 ± 2.50^{a}	23.58 ± 1.39^{b}	37.96 ± 0.80^{c}
NL10	0	6.75 ± 2.81^{a}	15.63 ± 4.45^{a}	17.69 ± 1.04^{ab}	28.01 ± 3.13^{b}
NL11	0	3.42 ± 2.97^{a}	29.88 ± 3.51^{b}	37.34 ± 3.92^{c}	43.98 ± 6.26^{d}
NL12	53.33 ± 5.77^{d}	66.14 ± 5.38^{c}	82.24 ± 6.74^{d}	95.83 ± 7.22^{e}	100^{f}
CV %	**27.6**	**21.5**	**14.1**	**12.1**	**7.6**
$LSD_{0.05}$	**4.48**	**5.81**	**6.91**	**7.65**	**5.96**

Note: Different letters within the same column represent significant differences at $p < 0.05$

Table 2. LC_{50} values of fungal isolates against *Oxya chinensis*

Isolates	LC_{50}	95% Fiducial limits		Slope	Intercept
		Lower	Higher		
4th-instar nymphs					
NL3	4.8×10^5	1.5×10^4	5.6×10^6	0.276	-1.569
NL4	1.6×10^5	2.0×10^3	1.3×10^6	0.287	-1.497
NL12	9.5×10^4	1.2×10^3	6.6×10^5	0.309	-1.538
Adults					
NL3	2.5×10^6	2.6×10^5	7.9×10^7	0.286	-1.830
NL4	1.5×10^6	1.2×10^5	3.3×10^7	0.280	-1.731
NL12	7.8×10^5	5.1×10^4	8.9×10^6	0.294	-1.731

Table 3. LT_{50} values of fungal isolates against *Oxya chinensis*

Isolates	LT_{50}	95% Fiducial limits		Slope	Intercept
		Lower	Higher		
4th-instar nymphs					
NL3	4.9	4.2	5.7	3.498	-2.402
NL4	4.4	3.8	5.1	3.632	-2.340
NL12	4.1	3.5	4.7	3.853	-2.346
Adults					
NL3	6.1	5.2	7.8	3.279	-2.578
NL4	5.9	5.0	7.5	3.180	-2.455
NL12	5.5	4.7	6.8	3.220	-2.394

Colonies of isolate NL4 were 35-40 mm in diameter after 7 days of incubation at 25°C on PDA. Colonies were white, with powdery texture and reverse side was also white in color. Based on microscopic observation, hyphae were branched and formed conidiogenesis cells. Conidia were single-celled or in-group, round to oval, and 2.0-3.0 µm. The macroscopic and microscopic characteristics of fungal isolate NL4 were similar to the typical characteristics of the genus *Beauveria*.

Colonies of isolate NL12 were 34-37 mm in diameter after 7 days of incubation at 25°C on PDA. Colonies were initially white or cream color, and then changed into greenish-yellow during sporulation. The reverse side was a pale cream color. Based on microscopic observation, conidia were single cells with cylindrical to an oval shape, and 3.0-3.5 µm. Macroscopic and microscopic characteristics of the fungal isolate NL12 were similar to the typical characteristics of the genus *Metarhizium*.

Identification of entomopathogenic fungal isolates using the ITS genes

To confirm the taxa of fungal isolates, ITS genes of three potential entomopathogenic fungi (NL3, NL4, NL12) were amplified and sequenced. The ITS genes of strains were sequenced with lengths ranging from 516 bp to 544 bp and had high similarity with the fungi of GenBank. ITS gene sequence of fungal isolate NL3 had 100% identity to *Aspergillus tamarii* L1 (accession number MT340979), ITS gene sequence of fungal isolate NL4 had 100% identity to *Beauveria bassiana* PL6111 (accession number AJ345089), and ITS gene sequence of fungal isolate NL12 had 100% identity to *Metarhizium anisopliae* PPRC (accession number MK862364). The phylogenetic tree was also shown in Figure 2. Identification of the fungal isolates based on morphology and ITS gene sequences revealed that isolate NL3 belonged to species *A.tamarii*, NL4 belonged to species *B. bassiana*, and NL12 belonged to species *M. anisopliae*.

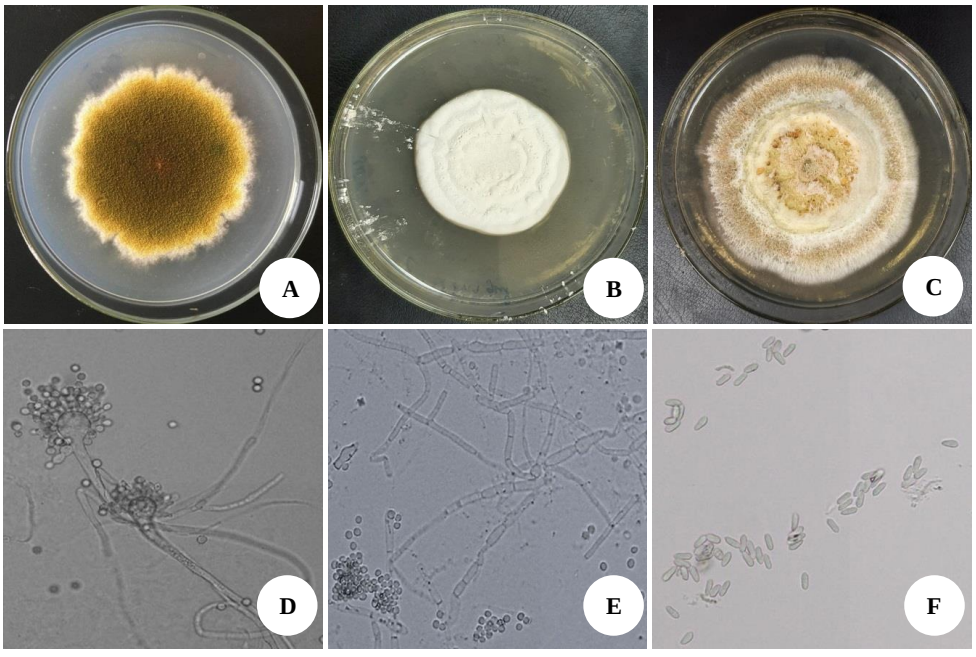

Figure 1. Colony morphology (A-C) and microscopic (D-F) morphology of the isolates NL3, NL4, NL12

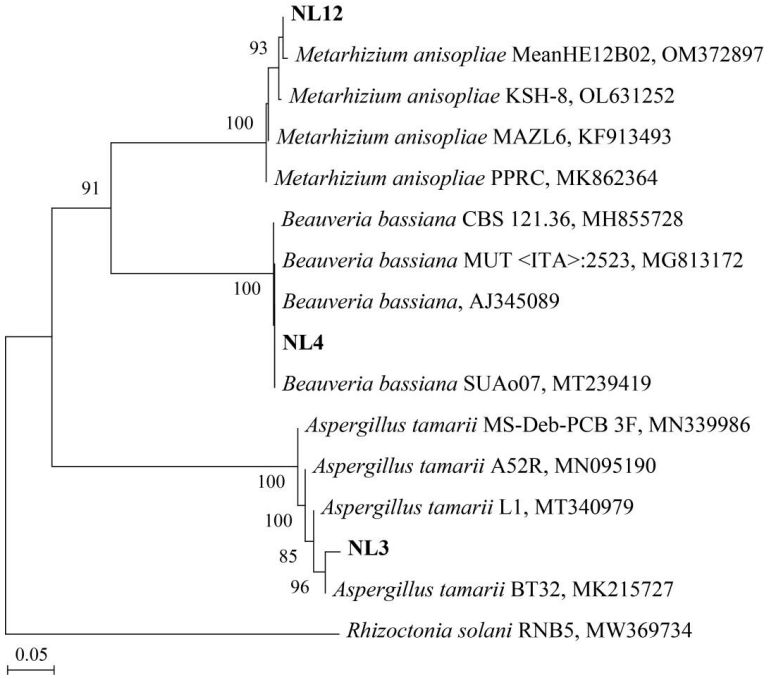

Figure 2. Phylogeny of ITS gene sequences of the fungal isolates (bold letters) and fungi from the National Center for Biotechnology Information (NCBI) GenBank. Bootstrap support values of branches greater than 75% are given above the corresponding branches

Discussion

Rice is a staple food for humans, especially for most people living in Asia; however, its yield is seriously threatened by insects and diseases (Papademetriou 2000). Among them, grasshoppers are one of the most abundant herbivorous insects of rice plants and cause severe damage to the rice crop. For many past years, the main control agents have been chemical insecticides. Unfortunately, chemical insecticides have potentially serious effects on the ecological environment, beneficial organisms, and human health (Ali et al. 2014). Therefore, biological control has become a particularly valuable agent for the control of grasshoppers and insects in agricultural production. In the present study, we isolated and screened potential entomopathogenic fungal strains from the dead grasshopper samples collected from rice fields in Thua Thien Hue against the 4th-instar grasshopper nymphs *O. chinensis* with high pathogencities (mortality rates > 85% after 15 days). LC_{50} values of three fungal isolates i.e. NL3, NL4, NL12 against 4th-instar nymphs and adults of *O. chinensis* ranged from 9.5×10^4 to 4.8×10^5 conidia/mL and 7.8×10^6 to 2.5×10^6 conidia/mL, respectively at 7 days. The LT_{50} values of NL3, NL4, NL12 isolates against 4th-instar nymphs and adults of *O. chinensis* ranged from 4.1 to 4.9 days and 5.5 to 6.1 days, respectively, at the concentration of 1×10^8 conidia/mL. Identification results showed that potential entomopathogenic fungi were *A. tamarii, B.bassiana,* and *M. anisopliae*.

Previous studies have reported that *B. bassiana* and *M. anisopliae* are potential entomopathogenic fungi to control different grasshoppers and locusts. Two fungal strains of *M. anisopliae* ICAR-NBAIR Ma-4 and ICAR-NBAIR Ma-35 showed 100% and 90% mortality, respectively against Aak grasshopper *P. pictus*, whereas two fungal strains of *B. bassiana* ICAR-NBAIR Bb-78 and ICAR-NBAIR Bb-45 also showed 40% and 14% mortality, respectively against *P. pictus*. Additionally, two fungal strains of *M. anisopliae* ICAR-NBAIR Ma-4 and ICAR-NBAIR Ma-35 showed 72.7% and 59.8% mortality, respectively against the eggs of grasshopper *P. pictus* (Ramanujam et al. 2021). In another study, two fungal strains of *B. bassiana* and *M. anisopliae* have been reported to show pathogenicity against the grasshopper *U. zebra* with mortality rates of 57.7% and 55.5%, respectively at a concentration of 5×10^6 spores/mL (Mohammadbeigi and Port 2015). Two fungal strains of *B. bassiana* G-07 and G-10 showed pathogenicity against grasshopper *Angaracris* sp. with mortality rates of 86.3-100% at concentrations of 2.1×10^8 and 2.1×10^9 conidia/mL, respectively (Otgonjargal et al. 2014), whereas two fungal strains of *M. anisopliae* Alberta 11S-1 and Alberta 6W-2 also showed pathogenicity against the grasshopper *M. bivittatus* with mortality rate > 90% after 7 treatment days (Adatia et al. 2010). *M. anisopliae* CQMa421 also showed pathogenicity againts the locust *L. migratoria* adults and nymphs with LT_{50} values of 6.0 and 5.0 days, respectively at the concentration of 1×10^8 conidia/mL, and LC_{50} (log10) values of 5.2 and 5.6, respectively after 10 days (Jiang et al. 2019). Unsurprisingly, *B. bassiana* and *M. anisopliae* have been developed as important bioinsecticides and used for commercial products for many years (Sandhu et al. 2017).

Several species of *Aspergillus* have also been reported as potential entomopathogenic fungi for the control of various grasshoppers and insects. The fungal strain of *A. oryzae* XJ-1 showed pathogenicity against locust *L. migratoria* with LC_{50} values of 3.3×10^8, 1.7×10^7, and 7.2×10^6 conidia/mL after 10, 13, and 15 treatment days, respectively (Zhang et al. 2015). Two strains of *A. tamari*

F2 and *A. flavus* F3 also showed pathogenicity against the bug *Elasmolomus pallens* with mortality rates of 62% and 90%, respectively after 10 days at a concentration of 1×10^8 conidia/mL (Umaru and Simarani 2020). Also, *A. flavus*, *A. fumigutus*, and *A.niger* showed pathogenicity against three grasshoppers *O. velox*, *P. pictus*, and *H. nigrorepletus*, whereas *A. tamari* showed pathogenicity against mosquitos *Aedes aegypti* and *Culex quinquefasciatus* (Baskar et al. 2020).

This is the first report of pathogenicity of fungi *A. tamarii*, *B. bassiana*, and *M. anisopliae* against the grasshopper *O. chinensis*. The present study confirms that entomopathogenic fungi are one of the important bioinsecticide sources for the protection of crops from grasshoppers and insects.

In conclusion the present study, 37 fungal isolates were isolated from the dead grasshoppers collected from rice fields in Thua Thien Hue province. The pathogenicity screening of 12 selected fungal isolates showed that mortality rates of 4th-instar grasshopper nymphs *O. chinensis* ranged from 21.99% to 100% after 15 exposure days at a concentration of 1×10^6 conidia/mL. Of these, three fungal isolates, namely NL3, NL4, NL12 showed the highest pathogenicity against the grasshopper *O. chinensis* with mortality rates of 87.73%, 89.81%, and 100%, respectively after 15 days. LC_{50} values of fungal isolates NL3, NL4, NL12 against 4th-instar nymphs and adults of *O. chinensis* ranged from 9.5×10^4 to 4.8×10^5 conidia/mL and 7.8×10^6 to 2.5×10^6 conidia/mL, respectively at 7 days. LT_{50} values of NL3, NL4, NL12 isolates against 4th-instar nymphs and adults of *O. chinensis* ranged from 4.1 to 4.9 days and 5.5 to 6.1 days, respectively, at a concentration of 1×10^8 conidia/mL. Identification of the potential entomopathogenic fungi based on morphology and ITS gene sequences showed that three potential entomopathogenic fungi were *A. tamarii*, *B. bassiana*, and *M. anisopliae*.

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