Egyptian Journal of
Biological Pest Control

RESEARCH

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Potential of Turkish *Beauveria bassiana* isolates for the management of the polyphagous planthopper, *Orosanga japonica* Melichar 1898 (Hemiptera: Ricaniidae)

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Abstract

Background: Entomopathogenic fungi are a potential biological control agent for many pests. In this study, 14 native *Beauveria bassiana* isolates were molecularly identified and assessed for their virulence and mortality against adults of the polyphagous planthopper *Orosanga japonica*, Melichar (Hemiptera: Ricaniidae) a polyphagous sap-feeding insect, under laboratory conditions.

Results: Isolates obtained from naturally infected adults *O. japonica* were molecularly identified as *B. bassiana* by sequencing the internal transcribed spacer and 26S large subunit of ribosomal DNA. In the bioassay, the radial growth and sporulation of isolates significantly differed ($P < 0.05$). Concentration–time bioassays demonstrated that all isolates had a lethal effect on adult *O. japonica* at the concentration of 1×10^8 conidia ml^{-1}. The lethal times LT_{50} and LT_{90} values for each *B. bassiana* isolate, which indicate the time required to kill *O. japonica*, ranged between 2.44–3.19 and 3.78–5.01 days, respectively, at 20 °C, while their values were 1.74–2.76 and 2.76–4.10 days, respectively, at 25 °C depending on the native isolates. The concentration–mortality response showed a statistically significant difference between some isolates *B. bassiana* tested against adult *O. japonica* individuals ($P < 0.05$). With an LC_{50} value of 2.29×10^6 conidia ml^{-1}, the lowest effective EPF isolate for *O. japonica* was KA-78-14. The isolates caused 100% adult mortality in *O. japonica* within five days, while the mortality in the control group was less than 10%.

Conclusions: These findings suggest that some *B. bassiana* isolates were more virulent on *O. japonica* and may play an important role in the biocontrol of *O. japonica* in Turkey.

Keywords: *Orosanga japonica*, Biocontrol, Entomopathogenic fungus, Median lethal time, LC_{50}, Molecular characterization

Background

The polyphagous planthopper, *Orosanga japonica* Melichar (Hemiptera: Ricaniidae), was introduced into the Eastern Black Sea region of Turkey, where hazelnut, tea, kiwifruit, blackberry, fresh bean, and corn are mainly cultivated (Ak et al. 2014). Since its introduction in 2007, *O. japonica* has caused significant damage to fruits, vegetables, and ornamental plants (Demir 2009).

The Eastern Black Sea region does not require intensive chemical control of diseases and pests that cause crop losses. Therefore, the largest tea production area in the region is relatively free from synthetic pesticides used in pest management activities. The population and distribution of this insect, however, has increased and become a significant problem in the region, which may lead to an increase in the use of pesticides (Altaş

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and Ak 2019). Conventional insecticides pose side effects, including the risk of chemical pollution, residual toxicity, insecticide resistance, and toxicity to non-target organisms. The adverse effects of insecticides on the environment and human health have led to the study of alternative methods. Therefore, environment-friendly strategies to control such important insect pests such as use of entomopathogenic fungi (EPFs) in biological control are one of the requirements for sustainable agriculture and food safety (Biryol et al. 2021).

EPFs are common natural enemies of arthropods worldwide and have attracted attention as potential biological control agents. Over 1,000 species belonging to around 100 genera of entomopathogens in the kingdom of fungi have been documented worldwide (Araújo and Hughes 2016). EPFs have been frequently studied due to their excellent potential to control insect pests in laboratory and field conditions (Biryol et al. 2022). *Beauveria bassiana* (Bals.-Criv) Vuill. (Ascomycota: Hypocreales) is one of the well-known EPFs and is wide spread throughout the world. It can infect and potentially control over 70 insect pests (Pedrini 2022). Also, *B. bassiana*-based products, accounting for about 34% of all commercial mycoinsecticides, are applied to manage agricultural pests' worldwide (Saruhan et al. 2014).

EPFs can be adapted to their environmental conditions such as certain climatic conditions and habitat types (Sayed and Dunlap 2019). Therefore, it is very important to isolate and identify native natural fungal strains on dead insects to control pests in the relevant areas. It is necessary to determine the species of local EPFs and their biocontrol effectiveness in a certain agroecosystem. Documenting EPFs on dead insects in the region, where *O. japonica* outbreaks occur, is important due to their ability to adapt to their environment. However, the pathogenic activity of native *B. bassiana* isolates against pests often differs among isolates, indicating the need to evaluate the potential of the Turkish *B. bassiana* isolates to manage *O. japonica* (Akıner et al. 2020).

Here, a study was conducted in 2019 to: (i) obtain several cadavers of naturally EPF-infected adult *O. japonica* in Artvin Province in the East Black Sea region of Turkey, (ii) identify the *Beauveria* species using DNA phylogeny based on ITS and LSU, and (iii) investigate *B. bassiana* isolates efficacy against adults of *O. japonica* under laboratory conditions. The overall objective was to determine if EPFs could be used to control *O. japonica*. The results would provide enough evidence to select more promising native *B. bassiana* isolates for pest control in orchards.

Methods

Sample collection and isolation of EPFs

From July to October 2019, infected *O. japonica* adult cadavers were collected from three kiwifruit orchards planted in Kemalpaşa District of Artvin Province, Turkey. First, the surface of the specimens was disinfected in 1% sodium hypochlorite solution for 1 min, rinsed with sterile water, and blotted dry. The dried specimens were placed in 9-cm-diameter Petri dishes containing potato dextrose agar (PDA; BD Difco, Sparks, MD, USA) medium supplemented with streptomycin sulfate (0.5 g l^{-1}) and incubated at 24 ± 1 °C for 7–10 days in the dark. Before identification studies, the single spore isolation of isolates was then performed using the serial dilution method. (Dhingra and Sinclair 1995). The EPF isolates were transferred to pieces (1 cm^2) of Whatman no. 1 filter papers overlaid on PDA. After colonization, the filter papers were dried and stored in the Eppendorf tubes at − 20 °C for further use and long-term storage (Erper et al. 2016). The strains of *B. bassiana* were deposited in the culture collection of the Mycology Laboratory of the Plant Protection Department, Faculty of Agriculture, Ondokuz Mayis University (Accession nos: KA-78-1-14).

Isolation of genomic DNA and molecular identification

Molecular tools, including phylogenetic analysis based on the internal transcribed spacer (ITS) and 26S large subunit (LSU), accurately identify EPF isolates. Conidial masses were gathered from the surface of 7-day-old PDA cultures and ground into powder in liquid nitrogen. According to the manufacturer's instructions, genomic DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). The quality and quantity of obtained DNA were measured spectrophotometrically by the A260/A280 ratio using a DS-11 FX + nano spectrophotometer (Denovix Inc., Wilmington, DE, USA). The DNA extract was diluted to 10 ng/µl and stored at − 20 °C for further analyses.

The ITS and LSU regions of ribosomal DNA were amplified with primer sets ITS1/ITS4 (White et al. 1990) and LR5/LR0R (Vilgalys and Hester 1990). The PCR mixture contained $1 \times$ PCR reaction buffer, 1.25-unit of Ampliqon TEMPase Hot Start DNA polymerase (Berntsen, Rodovre, Denmark), 0.2 mM of each dNTP, 0.4 µM of each primer, 10 ng of template DNA, and molecular grade water up to 50 µl. The PCR amplification was conducted at 95 °C for 15 min for an initial cycle to denature DNA and activate *Taq* DNA polymerase, followed by 95 °C for 45 s, annealing at 54 °C for 60 s, and extension at 72 °C for 90 s for 35 cycles; and a final extension at 72 °C for 10 min. The amplicons were

bidirectionally sequenced by Macrogen Inc., sequencing service (Seoul, Republic of Korea).

The DNA sequences were edited, and consensus sequences were manually estimated using Mega X computing platforms (Kumar et al. 2018). All sequences were compared against the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/genbank/) using the BLASTn algorithm, and deposited in GenBank. The isolates from this study, with additional reference isolates (Fig. 1) retrieved from the GenBank database, were aligned in the MAFFT v.7 online interfaces (Katoh and Standley 2013), http://mafft.cbrc.jp/alignment/server/) using default settings. A maximum likelihood (ML) tree of the combined ITS and LSU data set was inferred using the command-line version of IQ-TREE 1.6.7 (Nguyen et al. 2015) run on the CIPRES Science Gateway V 3.3. (Miller et al. 2010, https://www.phylo.org/), with ultrafast bootstrapping implemented with 1000 replicates. The resulting trees were analyzed and edited in FigTree v1.4.2 software. The ITS and LSU sequences of *Metarhizium anisopliae* strain CBS 662.67 were added as an-outgroup to facilitate the generation of consensus trees.

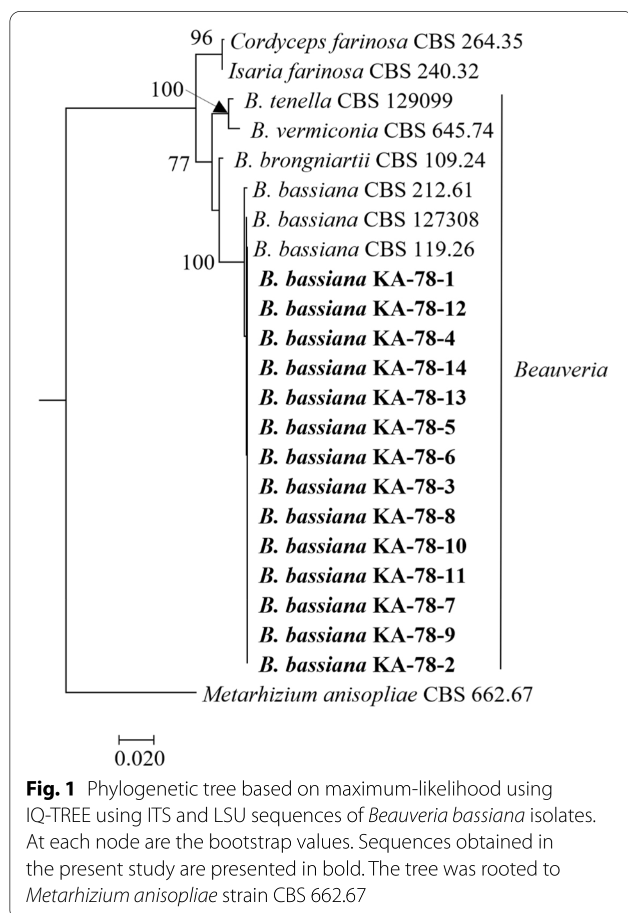

Fig. 1 Phylogenetic tree based on maximum-likelihood using IQ-TREE using ITS and LSU sequences of *Beauveria bassiana* isolates. At each node are the bootstrap values. Sequences obtained in the present study are presented in bold. The tree was rooted to *Metarhizium anisopliae* strain CBS 662.67

Determination of mycelial growth and conidiation

Mycelial growth and sporulation of 14 isolates were evaluated according to Cheng et al. (2016). Mycelial discs (5-mm-dia.) from 7 to 10-day-old fungal cultures were placed in the center of Petri dishes (9-cm-dia.) containing PDA, and the dishes were sealed by a parafilm and incubated at 25 ± 1 °C for two weeks. Their mycelial growth was measured on days 4, 8, 12, and 16 at two perpendicular radii of the colony, and their first day of sporulation was recorded. To determine the sporulation per unit area, at the end of the 16 days, an agar piece of 1 cm^2 for each isolate was cut from cultures where fungal growth occurs with a sterile scalpel and placed into 50-ml sterile polypropylene tubes. The conidia were released from the agar piece by shaking and dispersed in 20 ml of sterile distilled water containing Tween 20 (0.02%) (polysorbate, Merck Millipore KGaA, Burlington, USA). The conidia were then counted under a light microscope (DM1000, Leica Microsystems, Wetzlar, Germany) at 400× magnification using a hemocytometer, and the spore concentration per unit area was calculated. Four replicates were performed for each isolate, and the experiment was conducted once.

Insect culture

Adults of *O. japonica* were collected directly with a mouth aspirator from plants of kiwifruit (*Actinidia deliciosa*) cv. Hayward in 10-yr-old commercial orchards in the Artvin Province, on June. Insects were placed into a plastic box (30 × 40 × 25 cm) with a perforated lid for aeration, transported to the laboratory, and maintained in a climate chamber at 25 ± 1 °C for two days with a 16:8 h L:D photoperiod. Insect cultures were fed with fresh Acacia leaves (*Acacia* sp.), and after two days, healthy adults were gently selected for bioassays.

Inoculum of entomopathogen fungal isolates

Fourteen *B. bassiana* isolates were cultivated on PDA at 25 ± 1 °C for 15 days in darkness. Conidia were harvested with 100 ml of sterile distilled water containing Tween 20 (0.02%) and homogenized with a magnetic stirrer for 10 min. Then, mycelia were removed by filtering conidia suspensions through four layers of sterile cheesecloth. Conidial suspensions were counted under the DM1000 light microscope using a hemocytometer to calibrate five concentrations of each isolate (1×10^4, 10^5, 10^6, 10^7, and 10^8 conidia ml^{-1}) (Erper et al. 2016). To determine the conidial germination rate (%), 100 µl conidial suspension (1×10^4 conidia ml^{-1}) of each isolate was spread on PDA in 6-cm-diameter Petri dishes and incubated at 25 ± 1 °C. After 24 h, germinated conidia were counted by examining 100 conidia from four different areas in each dish, using the DM1000 light microscope at 400×

magnification. Conidia were regarded as germinated when they produced a germ tube, at least half of the conidial length. The experiment was three replicated for each isolate.

Concentration–time response bioassay

The concentration (1×10^8 conidia ml^{-1}) of all *B. bassiana* isolates prepared in sterile distilled water containing Tween 20 (0.02%) in advance was used to determine the concentration–time relationship. The bottoms of 500 ml plastic cups ($10.5 \times 8.5 \times 6.0$ cm), which 1.0% NaOCl disinfected, were covered by filter paper moistured with sterile-distilled water. Then, 10 adults of *O. japonica* were placed in the cups having fresh Acacia leaves. The concentration of each isolate was applied to adults (2 ml per cup) using a hand sprayer. Only sterile-distilled water containing Tween 20 (0.02%) was sprayed to control plastic cups, and the cups were incubated at either 20 ± 1 °C or 25 ± 1 °C at 75 ± 5% RH and 16:8 h L:D photoperiod. Inspections were made daily until individuals died in all the cups. Dead individuals were counted under a stereomicroscope (EZ4, Leica Microsystems, Wetzlar, Germany) at 40× magnification. The mortality rate was recorded daily, and dead individuals were removed from the cups. To determine the mycosis rate, evidence of *Beauveria* on which the fungal sporulation was observed on adult cadavers was verified by microscopic inspection after seven days (Boston et al. 2020). Three replicates of ten adults ($n = 30$) were used for each isolate, and the experimental design was completely randomized. Finally, the mean mortality rate was corrected for control mortality by Abbott's formula (Abbott 1925), and the percentage of mycosed cadavers was calculated.

Concentration–mortality response bioassay

Concentration-mortality bioassay trials were assayed using four isolates of *B. bassiana* based on their high efficacy on adults of *O. japonica* in the concentration–time bioassays. Five concentrations from 10^4 to 10^8 conidia ml^{-1} of the isolates were evaluated to determine the concentration–mortality relationship and the median lethal concentration (LC_{50}). *O. japonica* adults were exposed to these five concentrations of each isolate at 25 °C and sterile distilled water for control as the concentration–time response bioassay. The number of dead adults was documented for five consecutive days after applying conidial concentrations. The bioassays were conducted once, with three replications ($n = 30$). Finally, Abbott's formula was used to correct the mean mortality rate for control mortality, and the median LC_{50} values were calculated using probit analysis.

Statistical analyses

Since mortality rates exceeded 5% in pathogenicity tests, Abbott's formula was used to correct these data. Independent-time mortality data expressed (50% lethal time LT_{50}) and (LT_{90}) values from bioassays were calculated by fitting the data by using Logprobit method with Probit analysis program POLO-PLUS ver. 2.0 (Robertson et al. 1980). The LT_{50} and LT_{90} values of the isolates were compared using confidence intervals (95%), and the slopes of the regression lines were compared using standard errors. Data obtained from the present study were separately analyzed by one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant difference (HSD) at $P < 0.05$ using SPSS software version 14.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Identification of EPF isolates

The identification of 14 EPF isolates extracted from dead adults of *O. japonica* was confirmed using the BLASTn algorithm running on the NCBI website. The length of DNA fragments submitted to GenBank from the ITS and LSU regions was 547 and 979 bp for isolates, respectively. The sequences were deposited at GenBank under accession numbers listed in Table 1. BLASTn queries based on the ITS and LSU of *B. bassiana* isolates showed 99–100% nucleotide identity to those of the corresponding. Maximum-likelihood phylogenetic analysis based on combined ITS and LSU data confirmed the grouping of the isolates within the *B. bassiana* accessions (Fig. 1).

Table 1 Details of isolates of *Beauveria bassiana* obtained from *Orosanga japonica* adults

Isolate code	Place of origin	Accession nos	
		ITS	LSU
KA-78-1	Cumhuriyet/Kemalpaşa	MT102350	MT124984
KA-78-2	Cumhuriyet/Kemalpaşa	MT102351	MT124985
KA-78-3	Cumhuriyet/Kemalpaşa	MT102352	MT124986
KA-78-4	Cumhuriyet/Kemalpaşa	MT102353	MT124987
KA-78-5	Cumhuriyet/Kemalpaşa	MT102354	MT124988
KA-78-6	Uzunyalı/Kemalpaşa	MT102355	MT124989
KA-78-7	Uzunyalı/Kemalpaşa	MT102356	MT124990
KA-78-8	Uzunyalı/Kemalpaşa	MT102357	MT124991
KA-78-9	Uzunyalı/Kemalpaşa	MT102358	MT124992
KA-78-10	Selimiye/Kemalpaşa	MT102359	MT124993
KA-78-11	Selimiye/Kemalpaşa	MT102360	MT124994
KA-78-12	Selimiye/Kemalpaşa	MT102361	MT124995
KA-78-13	Selimiye/Kemalpaşa	MT102362	MT124996
KA-78-14	Selimiye/Kemalpaşa	MT102363	MT124997

Radial growth, sporulation and conidial germination of EPF isolates

The radial growth, sporulation and conidial germination of all the 14 *B. bassiana* isolates are shown in Table 2. There were generally significant differences between the growth rate and sporulation of the isolates ($P<0.05$). By the end of the 16th day, the radial growth of isolates KA-78-4, KA-78-7, and KA-78-11 was 2.25 cm as the highest value, followed by KA-78-14, KA-78-1, and other isolates. The growth rate of KA-78-5 was the lowest among all isolates. No positive relationship was found between the growth rate and sporulation of isolates, except for the KA-78-4, 78-2 and 78-10 isolates at a conidial concentration of 10^8 conidia ml^{-1}. The germination of the isolates varied from 97.50% (KA-78-4) to 92.8% (KA-78-5) (Table 2).

Concentration–time response

The results of concentration–time response analysis showed a significant difference between LT_{50} and LT_{90} of some *B. bassiana* isolates tested at 20 and 25 °C ($P<0.05$) (Table 3). At both temperatures, all the isolates at a concentration of (1×10^8 conidia ml^{-1}) were pathogenic to *O. japonica* adults. The LT_{50} and LT_{90} values, indicating the time required to kill *O. japonica* for each *B. bassiana* isolate, ranged between 2.44–3.19 and 3.78–5.01 days, respectively, at 20 °C, while it was 1.74–2.76 and 2.76–4.10 days, respectively, at 25 °C, depending on isolates. Considering the LT_{50} and LT_{90} values obtained under at 20 and 25 °C, it was found that some native isolates were more effective at 25 than 20 °C. The LT_{50} for isolate KA-78-2 against *O. japonica* was 2.87 days at 20 °C, whereas it was 1.74 at 25 °C. For isolate KA-78-3 against the pest, the LT_{90} value was 3.98 days at 20 °C, whereas it was 2.94 at 25 °C. Similarly, the LT_{50} values of the KA-78-6 isolate were 2.99 and 2.32 days at 20 °C and 25 °C, respectively. The LT_{90} value of the same isolate was 4.25 days at 20 °C, while the LT_{90} value for the same isolate was 3.21 days at 25 °C. The LT_{90} values for isolate KA-78-7 were 4.13 and 2.76 days at 20 °C and 25 °C, respectively.

Concentration–mortality response

Concentration–mortality response analysis revealed a statistically significant difference between *B. bassiana* isolates tested against adults of *O. japonica* ($P<0.05$) (Table 4). With an LC_{50} value of 2.29×10^6 conidia ml^{-1}, the least effective entomopathogenic isolate for *O. japonica* was KA-78-14. On the other hand, there were non-significant differences among the entomopathogenic effects of the other three isolates.

Mortality and mycosis

It was observed that the tested concentration (1×10^8 conidia ml^{-1}) of all the *B. bassiana* isolates on *O. japonica* adults under laboratory conditions began to cause mortality two days after the application, and the mortality increased with time at two different temperatures (20 and 25 °C). Also, the mortality caused by the isolates tested was generally variable (Figs. 2 and 3). *B. bassiana*

Table 2 Radial growth, sporulation, and conidial germination of *Beauveria bassiana* isolates

Isolate code	Radial growth (cm)				Initial sporulation time (days)	Sporulation (conidia cm$^{2-1}$)	Conidial germination (%)
	4 days	8 days	12 days	16 days			
KA-78-1	0.58 a*	1.28 cde*	1.68 dg*	2.18 abc*	3	1.01×10^8 bcd*	93.8
KA-78-2	0.52 ab	1.53 a	2.03 ab	2.13 a–e	3	1.26×10^8 ab	97.0
KA-78-3	0.52 ab	1.35 a–d	1.73 c–g	2.03 b–e	3	9.25×10^7 cde	94.5
KA-78-4	0.60 a	1.35 a–d	2.03 ab	2.25 a	3	1.46×10^8 a	97.5
KA-78-5	0.38 cd	1.13 e	1.48 g	1.93 e	3	9.30×10^7 cde	92.8
KA-78-6	0.33 d	1.38 a–d	1.88 b–e	2.13 a–e	3	9.76×10^7 bcde	94.5
KA-78-7	0.40 bcd	1.43 abc	1.93 a–d	2.25 a	3	1.06×10^8 bcd	95.5
KA-78-8	0.33 d	1.22 de	1.63 efg	1.95 de	3	5.83×10^7 f	94.3
KA-78-9	0.60 a	1.38 a–d	1.60 fg	2.03 b-d	3	6.66×10^7 ef	93.3
KA-78-10	0.53 ab	1.33 bcd	1.58 fg	2.15 a-d	3	1.20×10^8 abc	95.3
KA-78-11	0.53 ab	1.38 a–d	1.95 abc	2.25 a	3	1.04×10^8 bcd	95.0
KA-78-12	0.58 a	1.50 ab	1.78 b-f	2.00 cde	3	1.13×10^8 bc	96.0
KA-78-13	0.53 ab	1.40 a–d	1.90 a–d	2.13 a–e	4	7.96×10^7 def	93.3
KA-78-14	0.48 abc	1.38 a–d	2.15 a	2.23 ab	3	1.10×10^8 bcd	94.0

*According to Tukey's HSD test ($P<0.05$), the difference between the means followed by the same letter in each column is not significant

Table 3 Lethal times (LT_{50} and LT_{90}) for *Orosanga japonica* treated with the isolates of *Beauveria bassiana* (95% confidence limit) at concentration of 1 × 10^8 conidia ml^{-1}

Isolate code	20 °C			25 °C		
	LT_{50} (95% confi. limit)	LT_{90} (95% confi. limit)	X^2 (*P*-value)	LT_{50} (95% confi. limit)	LT_{90} (95% confi. limit)	X^2 (*P*-value)
KA-78-1	2.88 (1.95–3.79)	4.75 (3.66–13.47)	20.27	2.02 (1.25–2.74)	3.43 (2.56–7.90)	22.56
KA-78-2	2.87 (2.40–3.33)	4.14 (3.54–5.85)	10.78	1.74 (1.40–2.06)	2.80 (2.33–3.83)	7.83
KA-78-3	2.54 (2.13–2.90)	3.98 (3.41–5.28)	7.19	2.19 (2.08–2.29)	2.94 (2.78–3.18)	0.74
KA-78-4	2.55 (1.90–3.08)	3.88 (3.18–6.57)	14.63	1.95 (1.61–2.27)	3.02 (2.57–3.97)	7.19
KA-78-5	2.87 (1.46–4.36)	5.01 (3.60–5.51)	28.61	1.81 (1.48–2.13)	3.08 (2.59–4.10)	6.67
KA-78-6	2.99 (2.65–3.32)	4.25 (3.76–5.27)	6.25	2.32 (2.20–2.43)	3.21 (3.02–3.46)	3.51
KA-78-7	2.69 (2.10–3.22)	4.13 (3.41–6.65)	13.01	2.11 (2.00–2.20)	2.76 (2.60–2.99)	0.16
KA-78-8	2.45 (1.89–2.93)	3.79 (3.18–5.93)	11.95	2.12 (1.99–2.23)	3.03 (2.85–3.29)	3.94
KA-78-9	2.82 (2.48–3.16)	4.07 (3.58–5.12)	6.54	2.49 (–/–)	3.87 (–/–)	77.58
KA-78-10	2.59 (1.69–3.33)	3.96 (3.14–9.65)	21.61	2.36 (2.03–2.64)	3.31 (2.92–4.19)	6.24
KA-78-11	2.65 (2.21–3.04)	4.05 (3.46–5.53)	8.29	2.21 (1.88–2.48)	3.18 (2.80–4.00)	5.78
KA-78-12	2.44 (1.88–2.91)	3.78 (3.14–5.83)	11.94	2.07 (1.75–2.32)	3.00 (2.65–3.74)	5.41
KA-78-13	3.19 (2.51–3.90)	4.75 (3.89–8.76)	15.85	2.76 (2.29–3.19)	4.10 (3.50–5.75)	9.66
KA-78-14	2.46 (1.76–3.06)	4.17 (3.30–7.58)	14.65	2.34 (1.03–3.49)	3.74 (2.72–23.30)	5.03

Table 4 The median lethal concentration (LC_{50}) of adults of *Orosanga japonica* treated with four isolates of *Beauveria bassiana* applied at five concentrations

Isolate no	LC_{50} (conidia ml^{-1})	95% confidence interval		Slope ± SE	X^2
KA-78-4	2.95 × 10^5	4.72 × 10^3	3.21 × 10^6	0.387 ± 0.044	11.11
KA-78-8	2.32 × 10^5	1.10 × 10^5	4.44 × 10^5	0.425 ± 0.045	2.13
KA-78-12	1.91 × 10^5	7.67 × 10^4	4.06 × 10^5	0.361 ± 0.044	1.82
KA-78-14	2.29 × 10^6	9.69 × 10^5	6.07 × 10^6	0.305 ± 0.042	1.90

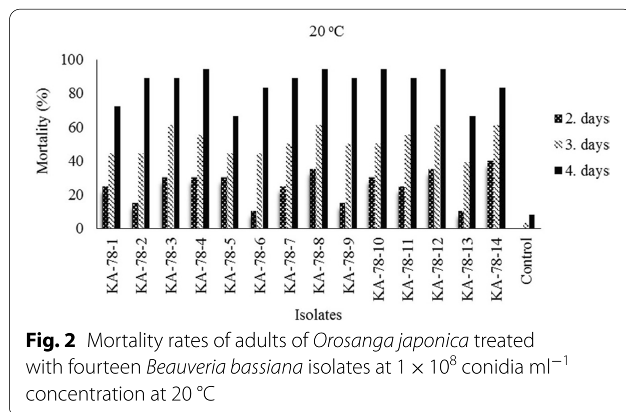

Fig. 2 Mortality rates of adults of *Orosanga japonica* treated with fourteen *Beauveria bassiana* isolates at 1 × 10^8 conidia ml^{-1} concentration at 20 °C

KA-78-4, KA-78-8, KA-78-10, and KA-78-12 isolates caused 94.44% mortality, four days after the application, while other isolates caused 66.67 up to 88.89% mortality at the tested concentration at 20 °C. However, isolates

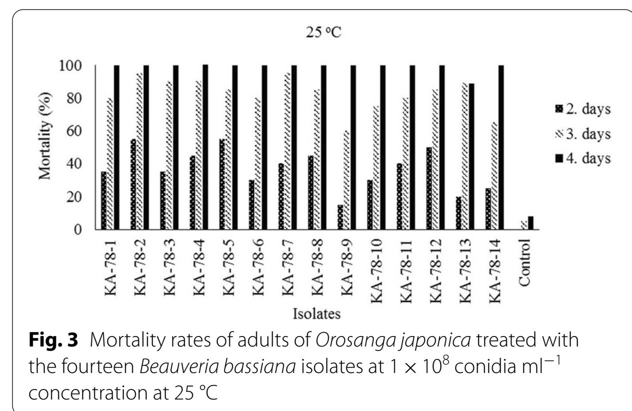

Fig. 3 Mortality rates of adults of *Orosanga japonica* treated with the fourteen *Beauveria bassiana* isolates at 1 × 10^8 conidia ml^{-1} concentration at 25 °C

KA-78-5 and KA-78-13 had the lowest mortality among all isolates under the same conditions (Fig. 2). Thirteen *B. bassiana* isolates resulted in 100% mortality four days after the treatment, except for the isolate KA-78-13 at 25 °C (Fig. 3). Mortality never exceeded 8.0% in controls of the treatments performed at 20 and 25 °C. Also, all the *B. bassiana* isolates caused about 100% mycosis rate in *O. japonica* adults in all treatments. Fungal vegetative growth was not detected in the control group.

Discussion

Over the last 15 years, the population of *O. japonica*, an invasive species in the Eastern Black Sea region, has been gradually increased, and the adults and nymphs of the pest became harmful to many wild and cultivated plants (Ak et al. 2015). It is important to investigate the

possibilities of using EPFs against this pest that spreads in the region.

The Eastern Black Sea region receives frequent rainfall and characterized by high humidity and low temperatures throughout the year. These environmental conditions are ideal for the development of EPFs such as *B. bassiana* and *Lecanicillium muscarium* Zare and Gams (Ascomycota: Hypocreales) (Akıner et al. 2020). Conidia of EPFs need moisture for germination and subsequent sporulation; some even require high humidity to initiate infection. Rain also plays an important role in spreading EPFs among insect individuals (Goettel et al. 2005). The present study showed that all native isolates of EPF isolated from adult cadavers of the pest collected from kiwifruit orchards in Artvin Province (Eastern Black Sea region) were identified as *B. bassiana* based on the rDNA-ITS sequencing. Akıner et al. (2020) reported that the natural mortality rates caused by *B. bassiana* in the surveys conducted in the areas correct as infested by *O. japonica* in Rize Province located in the same region were approximately 80–95% and 80–98% in 2018 and 2019, respectively. All tested isolates caused significant mortality against *O. japonica* adults under laboratory conditions. Several previous studies indicated that the EPF were more likely to have ecological compatibility with pests due to their geographical locations and habitats types (Bidochka et al. 2002; Akıner et al. 2020).

The efficiency of an EPF isolate depends on temperature, relative humidity, host species, host life stage, and duration of incubation (Bugti et al. 2018). It is known that the isolates can be adapted to temperatures occurring in the origin location from which they were isolated. In general, optimum growth and germination rates in artificial environments are about 25 °C for fungi. The fact that *B. bassiana* species can develop in a wide temperature range, such as 8–35°C (Fargues et al. 1997). In addition, Bugeme et al. (2008) showed that for *B. bassiana* growth 25 °C was better than at 20 °C. In the present study, the native *B. bassiana* isolates caused deaths in adults of the pest at both 20 °C and 25 °C. The mortality rate of some *B. bassiana* isolates (KA-78-4, KA-78-8, KA-78-10, and KA-78-12) ~ reached 94%, while mortality rates of the remaining ten isolates ranged between ~67–89% at the tested concentration at 20 °C; on the other hand, the mortality rate of almost all isolates used in the trial resulted in 100% mortality, four days after the treatment at 25 °C at (1×10^8 conidia ml^{-1}) concentration. These findings are in line with those of several previous studies. Sevim et al. (2013) reported that among 13 EPF isolates, *B. bassiana* isolate KTU-24, which was obtained from *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Thaumetopoeidae), showed the highest mortality against adults and nymphs of *Corythucha ciliata* with 86% within 14 days after inoculation, at 1×10^7 conidia ml^{-1} concentration, while the same isolate caused 100% mortality on adults and nymphs of *C. ciliata* at 1×10^8 conidia ml^{-1} concentration.

In the present study, the LT_{50} and LT_{90} values of the isolates tested varied between 1.74–2.76 and 2.76–4.10 days, respectively, at 25 °C, and between 2.44–3.19 and 3.78–5.01 days, respectively, at 20 °C. Considering the LT_{50} and LT_{90} values, *B. bassiana* isolates were slightly more effective at 25 than at 20 °C, which put forward some *B. bassiana* isolates as significant candidate organisms for controlling *O. japonica* in the origin region of the country. Akıner et al. (2020) found that the lowest LT_{50} values for *B. bassiana* isolate-1 and isolate-2 were 2.92 and 2.56 days, respectively, for nymphs using the leaf dipping method at (1×10^6 conidia ml^{-1}). In addition, EPF isolates with a high virulence and good growth characteristics are the basis in pest biocontrol. Overall, mortality and median lethal times (LT_{50} and LT_{90}) can indicate the pathogenicity of strains on harmful pests. The highest mortality and the lowest LT_{50} value caused by an EPF isolate indicate high virulence and potential for use in biological control (Wekesa et al. 2005).

The mycosis rate of a fungal isolate is an important factor for dissemination and secondary recycling of the fungus in the field (Goettel et al. 2005). In the present study, the native *B. bassiana* isolates were screened against *O. japonica* adults to find possible fungal biocontrol agents that could be utilized against this pest. At the end of mycosis tests, it was detected that the *B. bassiana* isolates tested can infect *O. japonica* adults and can produce conidiophores and conidia on the surface of the cadavers. Accordingly, fungal sporulation on dead insects in nature can form a source of inoculum for live insects, and EPF conidia may play an important role in horizontal transmission between pest male and female individuals and disease development in the field. In other words, EPF can be used to decrease pest populations as horizontal transmission strategies (Kimiaei et al. 2022).

There is no general rule that any given stage of development of an insect is always more susceptible to EPF (Goettel et al. 2005). In most EPF, there is a differential virulence towards the life stages of insects, and not all stages in an insect's life cycle are equally susceptible to fungal infection. Sometimes, the pest's different life stages (larvae, nymph or adult) against EPF can be more susceptible than another life stage. Contrary to obtained findings, Akıner et al. (2020) reported that *O. japonica* nymphs exposed to two native *B. bassiana* isolates obtained from dead *O. japonica* specimens were more susceptible than to the adults. In another study, Tarasco and Triggiani (2006) reported that especially *B. bassiana*

is potentially an effective biological control agent against overwintering adults of *C. ciliata* in the field.

Conclusions

Native 14 *B. bassiana* isolates from *O. japonica* cadavers were obtained. Phylogenetic analysis based on ITS and LSU confirmed the identification and grouped these EPF isolates with reference *B. bassiana* isolates derived from GenBank. No genetic variation was determined among the isolates obtained in this study. These loci in the ribosomal DNA can be used for the accurate identification of *B. bassiana* isolates. Also, the adulticidal activities of the *B. bassiana* isolates against *O. japonica* adults under controlled laboratory conditions were assessed. The native isolates tested could be used as possible biocontrol agents against the *O. japonica*. Further studies are required to determine whether the native *B. bassiana* isolates could be successfully used to biocontrol of nymphs and adults of *O. japonica*, and horizontal transmission between adults and nymphs in the field. Moreover, the side effects of these isolates should also be investigated in the region infested with *O. japonica* and have plentiful beneficial organisms.

Abbreviations
O. japonica: *Orosanga japonica*; *B. bassiana*: *Beauveria bassiana*; *C. ciliata*: *Corythucha ciliata*; EPF: Entomopathogenic fungi; LC_{50}: Median lethal concentration; ITS: Internal transcribed spacer; LSU: Large subunit; EF1-α: Elongation factor 1-alpha.

Acknowledgements
Not applicable.

Author contributions
IE, KA and EY participated in setting the work planning and executing the experimental work. IE was provided entomopathogenic fungal isolates for study. MT analyzed the all data (statistical analyses) in study. IE, GO and MA and are the contributors in writing the manuscript. GO and MT revised the manuscript. All authors read and approved the final manuscript.

Funding
No funding.

Availability of data and materials
The datasets used and analyzed during the current 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.

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Received: 30 May 2022 Accepted: 22 August 2022
Published online: 29 August 2022

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