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Evaluation of entomopathogenic fungi against the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae)

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Abstract: The sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae), is one of the most destructive pests of plane trees (*Platanus* spp.) worldwide. This pest is a major nuisance in Europe since plane trees are very popular in parks. Thirteen entomopathogenic fungal strains including 4 isolates of *Beauveria bassiana*, 2 isolates of *Beauveria pseudobassiana*, 6 isolates of *Metarhizium anisopliae*, and 1 isolate of *Isaria fumosorosea* were screened against adults and nymphs of *C. ciliata* under controlled laboratory conditions in order to test their efficacy and search for an effective and safe biocontrol agent strategy. Each isolate was applied with a conidial concentration of 1×10^7 conidia mL^{-1} to adults and nymphs of the pest. *B. bassiana* isolate KTU – 24 showed the highest mortality for both adults and nymphs with 86% within 2 weeks after inoculation. This isolate also caused the highest mycosis for adults and nymphs with 83% and 80%, respectively. Mortalities of the other fungal isolates ranged from 43% to 86% and from 36% to 73% for adults and nymphs. Therefore, *B. bassiana* KTU – 24 was selected for further dose mortality tests based on its high virulence and mycosis value. Dose–response mortality bioassays using 5 different concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia mL^{-1}) were performed to determine the lethal concentration (LC_{50}) of this isolate. Based on probit analysis, the LC_{50} values of isolate *B. bassiana* KTU – 24 were calculated as 5.51×10^5 and 3.96×10^5 conidia mL^{-1} against adults and nymphs, respectively. Consequently, *B. bassiana* KTU – 24 appears to be a promising candidate for further investigations as a biocontrol agent against *C. ciliata*.

Key words: Sycamore lace bug, *Beauveria bassiana*, bioassay, virulence, microbial control

1. Introduction

The sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae), is one of the most important pests of plane tress, especially *Platanus orientalis* (L.) (Proteales: Platanaceae), which is a popular shade and ornamental tree (Barnard and Dixon 1983; Hui-Lin 1996; Mutun 2009; Ju et al. 2011). In Europe, this pest was first observed in 1964 in Italy, and it has spread throughout central and southern Europe (d'Aguilar et al. 1977). In Turkey, it was first detected in Bolu Province in 2007 (Mutun 2009), and it has spread rapidly to other provinces such as Tekirdağ (Aysal and Kıvan 2011). Both adults and nymphs of *C. ciliata* feed on the underside of leaves and produce small chlorotic stipplings on the upper leaf surface (Halbert and Meeker 1998). Their injury reduces photosynthesis and respiration of host plants and also affects the aesthetic value of the trees. As a result, foliage becomes bronzed and leaves may fall earlier, in late summer (Halbert and Meeker 1998). Several years of severe damage by *C. ciliata*, combined with the effects of other environmental factors, may kill the trees (Barnard and Dixon 1983). Moreover, this pest is known to be associated with plant pathogenic fungi such as *Ceratocystis fimbriata* (Ellis & Halsted) and *Apiognomonia veneta* (Sacc. & Speg.), which can cause decline and death in combination (Malumphy et al. 2007). In addition to damaging trees, the sycamore lace bug can become a major nuisance in Europe, as the plane tree is a very popular shade tree in parks and on streets. This pest is particularly bothersome due to being found in large numbers in open air bars and cafes that are shaded by sycamore trees, and they may also invade homes in large numbers (Maceljski 1986).

There are a few control methods for *C. ciliata*, such as spraying a strong stream of water to dislodge young nymphs from leaves or inspecting leaves every 2 weeks during the growing season (especially if there was damage in previous season); however, they are not effective (Kluepfel and Scott 2011). In addition, there are some natural enemies of the pest such as assassin bugs, minute pirate bugs, lacewings, spiders, and predaceous mites, but they are not currently

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being used in any biocontrol program (Malumphy et al. 2006; Scheffler and Goodwin 2008). A wide variety of insecticides such as organophosphates, synthetic pyrethroids, imidacloprid, thiamethoxam, or acetamiprid and various methods of application (e.g., foliar sprays, trunk injections, soil treatments) are applied for use against the sycamore lace bugs; however, these insecticides are costly, and efficacy is often marginal (Halbert and Meeker 1998; Kim et al. 2000; Ju et al. 2009). Furthermore, chemical insecticides also have undesirable side effects on the environment and humans (Sezen and Demirbag 2006). Despite all of these control methods, this pest still continues to be a major pest of plane trees.

Biological control of pests with entomopathogenic fungi is an attractive alternative to the use of conventional pesticides, mainly because these fungi are safer for plants, animals, and the environment (Khetan 2001). Among many entomopathogenic fungi, much research effort has been placed on the development of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* sensu lato as biological control agents (for inundation and inoculation biological control) to be applied in agriculture and forestry in temperate regions (Meyling and Eilenberg 2007). Up to now, the entomopathogenic fungus *B. bassiana* has been extensively used for the control of many important pests of various crops around the world and it has been tested on different target insects, both under laboratory and field conditions (Campbell et al. 1985; Leathers and Gupta 1993; Lacey et al. 1994; Todorova et al. 2002; Askary and Yarmand 2007; Cottrell and Shapiro-Ilan 2008; Tunaz et al. 2008; Sevim et al. 2010a; 2010b; 2010c; Tanyeli et al. 2010). Entomopathogenic fungi differ from other insect pathogens since they are able to infect through the host's integument. Therefore, ingestion is not necessary and infection is not limited to chewing insects. They are unique for controlling insect pests that feed by sucking plant or animal juices (Fuxa 1987; Lacey and Goettel 1995; St. Leger and Roberts 1997; Roy and Cottrell 2008; Sevim et al. 2012a).

Although *C. ciliata* is an important pest species worldwide, there are only a few studies evaluating biocontrol of this pest by using entomopathogenic fungi. Since *C. ciliata* adults and nymphs feed by sucking plant sap from the undersides of leaves, entomopathogenic fungi should be effective candidate organisms for control of this pest. The main aim of the present study was to test the effectiveness of 13 isolates of entomopathogenic fungal strains against *C. ciliata* under laboratory conditions. The results presented here can be beneficial in further biological control programs against *C. ciliata*.

2. Materials and methods

2.1. Collection of insects

C. ciliata adults and nymphs were collected from infested *P. orientalis* trees in the vicinity of Trabzon, Turkey, between June and August 2011. They were either carefully taken from undersides of leaves by a soft fine-tipped paintbrush or caught by a sweep net (flying adults). Insect samples were placed into plastic boxes (20 mm) with ventilated lids and freshly collected plane leaves as food. Afterwards, they were transported to the laboratory. Healthy adults and nymphs were acclimated for 2 days to the laboratory conditions. After 2 days, healthy adults and last instar nymphs were separated and used for bioassays.

2.2. Fungal isolates

Fungal isolates were obtained from stock cultures at the Microbiology Laboratory of the Department of Biology at Karadeniz Technical University, Trabzon, Turkey. A total of 13 entomopathogenic fungi including *Beauveria bassiana* (Bals.) Vuill. (4 isolates), *B. pseudobassiana* S.A. Rehner & R.A. Humber (2 isolates), *Metarhizium anisopliae* sensu lato (6 isolates), and *Isaria fumosorosea* (Wize) (1 isolate) were used for bioassays (Table 1). Both *B. bassiana* and *I. fumosorosea* isolates were morphologically and molecularly identified (Sevim et al. 2010a, 2010c). However, *M. anisopliae* isolates were only identified by morphology and were therefore referred to as *M. anisopliae* sensu lato. All isolates were known to be highly virulent against *Tenebrio molitor* (Linn.) (Coleoptera: Tenebrionidae) larvae under laboratory conditions (Sevim et al. 2012b). Isolates were cultivated on PDAY (potato dextrose agar + 1% yeast extract; Merck, Darmstadt, Germany) for 4 to 5 weeks at 28 °C. The fungi were stored at 4 °C until needed for the bioassay experiments.

2.3. Preparation of spore suspensions

Fungal isolates were propagated from a single colony to obtain pure cultures. Therefore, 100 µL of spore suspension of stock fungal cultures (1×10^6 conidia mL^{-1}) was plated on PDAY and incubated at 25 °C for 4 to 5 days under a 12-h L / 12-h D photoperiod. After 4 to 5 days, a single colony was cut out and transferred to another fresh PDAY plate and incubated at 25 °C for 4 to 5 weeks until plates were fully overgrown. Conidial suspensions of fungal isolates were prepared by adding 15 mL of sterile 0.01% Tween 80 (Applichem) into the 4-week-old petri dishes and gently scraping the surface of the cultures with a sterile bent glass rod to dislodge the conidia from the surface of the agar plates. The conidial suspensions were filtered through 2 layers of sterile muslin into 50-mL sterile plastic tubes (Falkon) to remove mycelium and agar pieces. The obtained conidial suspensions were vortexed for 5 min for homogenization. Finally, the spore concentration was determined with a Neubauer hemocytometer and adjusted to the desired concentrations.

Table 1. Fungal isolates and their sources.

No.	Species	Isolates	Locality	Source
1	*Beauveria bassiana*	KTU – 7	Yomra, Trabzon	Soil
2	*B. bassiana*	KTU – 24	Samsun	*Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Thaumetopoeidae)
3	*B. bassiana*	KTU – 25	Ünye, Ordu	Soil
4	*B. bassiana*	KTU – 57	Gümüşhane	*Rhynchites baccus* (L.) (Coleoptera: Rhynchitidae)
5	*B. pseudobassiana*	KTU – 53	Gümüşhane	Soil
6	*B. pseudobassiana*	KTU – 55	Bayburt	Soil
12	*Metarhizium anisopliae*	KTU – 2	Ardeşen, Rize	Soil
8	*M. anisopliae*	KTU – 21	Borçka, Artvin	Soil
7	*M. anisopliae*	KTU – 27	İkizdere, Rize	Soil
9	*M. anisopliae*	KTU – 40	Akçaabat, Trabzon	Soil
10	*M. anisopliae*	KTU – 51	Gümüşhane	Soil
11	*M. anisopliae*	KTU – 60	Gümüşhane	Soil
13	*Isaria fumosorosea*	KTU – 42	İkizdere, Rize	Soil

Conidial viability was determined by enumerating the percentage of the germinated conidia 24 h after spreading 100 µL of conidial suspensions (1×10^6 mL^{-1}) on PDAY medium. Plates were incubated at 25 °C under a 12-h L / 12-h D photoperiod. Conidia were considered to have germinated if the germ tube was longer than the diameter. Isolates with higher germination rates of 95% were used for bioassay experiments.

2.4. Screening tests

Screening tests were performed on both adults and the last instar nymphs of *C. ciliata*. The spore concentration of 1×10^7 conidia mL^{-1} was used in the initial screening bioassay. Sterile distilled water with Tween 80 (0.01%) was used as the control. Freshly collected plane leaves were provided as food for both adults and nymphs. For bioassay, 10 adults or nymphs, a mix of males and females, were carefully taken with a soft paintbrush onto aluminum foil and the conidial suspensions (2 mL) were applied with a sterile sprayer for 10 s. They were carefully placed on the underside of the small plane leaves in a plastic box (20 mm) with a ventilated lid. The same number of adults or nymphs were used for the control and sprayed only by sterile, distilled water with Tween 80 (0.01%). Both adult and nymph experiments were carried out with 10 individual per replicate and fungal isolate, and all experiments were repeated 3 times on different occasions. All treated and untreated adult and nymph were kept in rearing boxes at 25 °C for 2 weeks under a 12-h L / 12-h D photoperiod. Freshly collected detached plane leaves were provided every day during the 2 weeks. At the end of the incubation period, dead insects were counted and cadavers were immediately surface-sterilized with 1% sodium hypochlorite for 30 s, followed by 3 rinses with sterile distilled water. They were placed on wet filter paper in sterile plastic petri dishes (15 mm), sealed with Parafilm and incubated at 25 °C to induce sporulation on the cadavers. Finally, mortality data were corrected using Abbott's formula (Abbott 1925) and the percentage of mycosed cadavers was calculated.

2.5. Dose–mortality response test

Dose–mortality response test studies were conducted using *B. bassiana* strain KTU – 24 based on its high efficacy on both adults and nymphs of *C. ciliata* in the initial screening test. Ten adults and the last instar nymphs were treated with 5 different conidial concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia mL^{-1}) in 3 replicates. The spore concentrations (2 mL) were applied using a small sprayer (100 mL). After that, adults and nymphs were separately put into plastic boxes (20 mm) with ventilated lids including a small plane leaf. Mortality of adults and nymphs were checked every day for 14 days after inoculation of conidial concentrations. Finally, mortality data were corrected using Abbott's formula (Abbott 1925) and the lethal concentration (LC_{50}) value was calculated using probit analysis.

2.6. Statistical analysis

Mortality data were corrected by Abbott's formula (Abbott 1925) and percentages of mycosed cadavers were calculated. The data were subjected to analysis of variance (ANOVA) followed by post-hoc least significance difference (LSD) multiple comparison tests to compare test isolates with each other and the control group with respect to mortality and mycosis ($P < 0.05$). Likewise, the data from the dose–mortality response test were subjected to ANOVA followed by post-hoc LSD multiple comparison tests to determine differences among different concentrations ($P < 0.05$). Chi-square analysis was used to determine the difference between adults and nymphs in terms of susceptibility to fungal isolates ($P < 0.05$). Finally, LC_{50} values were determined by probit analysis. Computations for all experiments were performed using SPSS 15.0.

3. Results

3.1. Screening tests

Fungal isolates caused different mortality in both adults and nymphs (Figures 1 and 2). In the case of adult mortality, all isolates produced significantly different mortality ($F = 4.17$, $df = 13, 28$, $P = 0.001$), but there was no difference between the control and KTU – 25, KTU – 40, or KTU – 51 ($F = 4.17$, $df = 13, 28$, $P = 0.001$). The highest mortality was obtained from *B. bassiana* KTU – 24 and *M. anisopliae* KTU – 60 with 86 ± 5.77% mortality values for both 14 days after inoculation. The second highest mortality was recorded from *M. anisopliae* KTU – 2 with 83% mortality within the same period (Figure 1). All isolates displayed different mycosis values ($F = 24.37$, $df = 13, 28$, $P < 0.0001$), and there was no significant difference between the control and KTU – 53, KTU – 21, KTU – 55, KTU – 40, or KTU – 51 ($F = 24.37$, $df = 13, 28$, $P < 0.0001$). The highest mycosis value was obtained from *B. bassiana* KTU – 24 with 83% (± 5.77), which was different from all other treatments and the control ($F = 24.37$, $df = 13, 28$, $P < 0.0001$) (Figure 1).

In the case of nymph mortality, the fungal isolates produced different mortality in comparison to each other ($F = 5.27$, $df = 13, 28$, $P = 0.00011$), and there was no significant difference between the control and KTU – 53, KTU – 25, KTU – 55, KTU – 40, KTU – 51, or KTU – 7 ($F = 5.27$, $df = 13, 28$, $P = 0.00011$). The highest mortality was recorded from *B. bassiana* KTU – 24 with 86 ± 5.77% 14 days after inoculation. The second highest mortality was obtained from *M. anisopliae* KTU – 27 with 80% within the same period (Figure 2). All isolates produced different mycoses in comparison to each other ($F = 33.87$, $df = 13, 28$, $P < 0.0001$), and there was a significant difference between the control and KTU – 42, KTU – 24, KTU – 57, and KTU – 7 ($F = 33.87$, $df = 13, 28$, $P < 0.0001$). *B. bassiana* KTU – 24 produced the highest mycosis value with 80 ± 10%, which was different from all other treatments and the control ($F = 33.87$, $df = 13, 28$, $P < 0.0001$). *B. pseudobassiana* KTU – 57 produced the second highest mycosis value with 63 ± 20.81% (Figure 2).

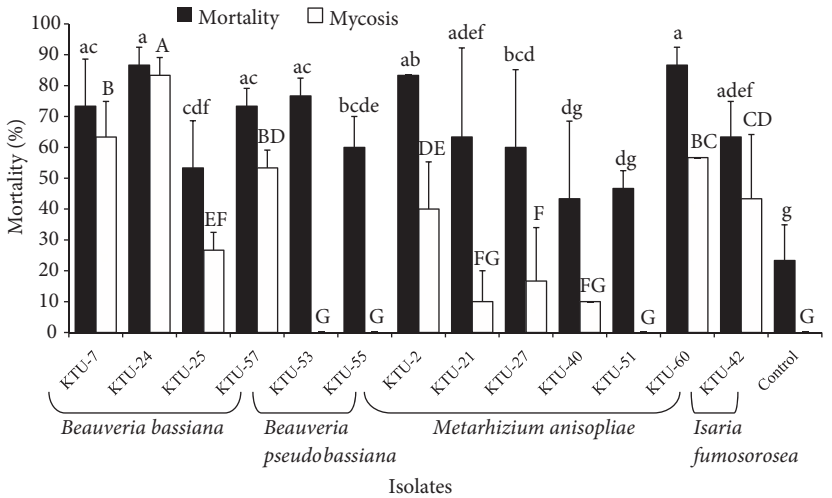

Figure 1. Mortality of *C. ciliata* adults (%) after application of 13 entomopathogenic fungal isolates within 14 days after application of 1×10^7 conidia mL^{-1}. Mortality data were corrected according to Abbott's formula (Abbott 1925). Different uppercase and lowercase letters represent statistically significant differences among mortality and mycosis, respectively, between treatments according to LSD multiple comparison test ($P < 0.05$). Bars show standard deviation. KTU – 7, KTU – 24, KTU – 25, and KTU – 57: *B. bassiana*; KTU – 53 and KTU – 55: *B. pseudobassiana*; KTU – 2, KTU – 21, KTU – 27, KTU – 40, KTU – 51, and KTU – 60: *M. anisopliae*; KTU – 42: *I. fumosorosea*; control: Tween 80 (0.01%).

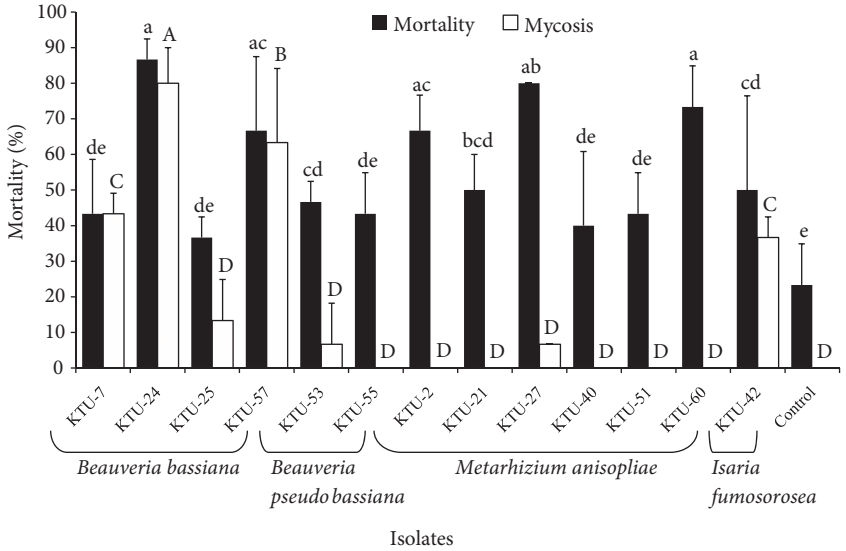

Figure 2. Mortality of *C. ciliata* nymphs (%) after application of 13 entomopathogenic fungal isolates within 14 days after application of 1×10^7 conidia mL^{-1}. Mortality data were corrected according to Abbott's formula (Abbott 1925). Different uppercase and lowercase letters represent statistically significant differences among mortality and mycosis, respectively, between treatments according to LSD multiple comparison test ($P < 0.05$). Bars show standard deviation. KTU – 7, KTU – 24, KTU – 25, and KTU – 57: *B. bassiana*; KTU – 53 and KTU – 55: *B. pseudobassiana*; KTU – 2, KTU – 21, KTU – 27, KTU – 40, KTU – 51, and KTU – 60: *M. anisopliae*; KTU – 42: *I. fumosorosea*; control: Tween 80 (0.01%).

There was no significant difference between adult and nymph mortality according to the chi-square test ($P > 0.05$). There was also no significant difference between adult and nymph with respect to mycosis ($P > 0.05$).

3.2. Dose–mortality response test

B. bassiana KTU – 24 was selected for dose application based on its high virulence on *C. ciliata* adults and nymphs. In the dose–mortality response tests of *B. bassiana* isolate KTU – 24, adult mortality reached 100% within 14 days after application of the conidial concentration of 1×10^8 conidia mL^{-1} and there was a significant difference among concentrations ($F = 126.56$, $df = 5, 12$, $P < 0.0001$) (Figure 3). Probit analysis was used to determine the LC_{50} value (Table 2). The LC_{50} of this fungus against the adults within 14 days after treatment was determined to be 5.51×10^5 conidia mL^{-1}. Nymph mortality also reached 100% within 14 days after application of the 1×10^8 conidia mL^{-1} concentration, while mortality in the controls was 13 ± 5.77% (Figure 4). The results show that the concentration of conidia affected the mortality of nymphs differently ($F = 43.27$; $df = 5, 12$; $P < 0.0001$). The LC_{50} of this fungus against the adults 14 days after treatment was determined to be 3.96×10^5 conidia mL^{-1} (Table 2).

4. Discussion

The chemical insecticides used against insect pests in agriculture and forestry have contributed undesirable side effects to animals, plants, and the environment. The increasing concern about these side effects has necessitated a change in strategies to manage insect pests in an ecologically acceptable manner. These concerns encouraged scientists to search for biopesticides such as microbial pesticides to provide sustainable control methods. Entomopathogenic fungi are an attractive, effective, and environmentally safe alternative to control many important pest species because they are safer for the environment. *C. ciliata* is an important pest species of plane trees worldwide; however, studies related to microbial control agents of the pest are limited. In this study, different entomopathogenic fungi were screened against *C. ciliata* adults and nymphs to find possible fungal biocontrol agents that could be utilized against this pest. We showed that the tested fungal isolates can infect *C. ciliata* adults and nymphs and are able to produce conidiophores and conidia on the surface of the cadavers.

B. bassiana KTU – 24 caused 86% mortality, which was higher than the other fungal treatments applied against both *C. ciliata* adults and nymphs. Balarin and Maceljski (1986a, 1986b) investigated natural enemies of this pest and found that *B. bassiana* caused a 10%–15% infection rate

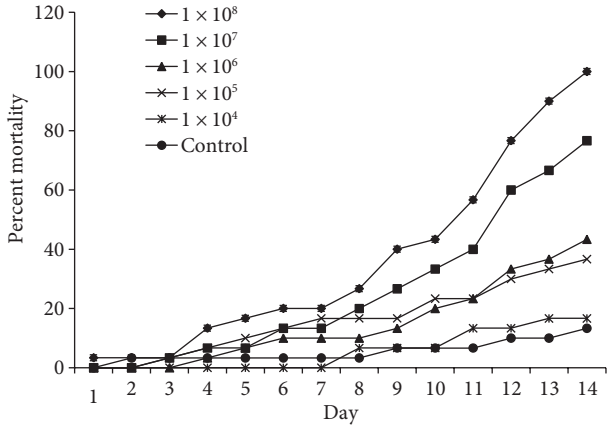

Figure 3. Cumulative mortality of *Corythucha ciliata* adults after application of 5 different doses of the spore concentration of *Beauveria bassiana* KTU – 24. Concentration unit is conidia mL^{-1}.

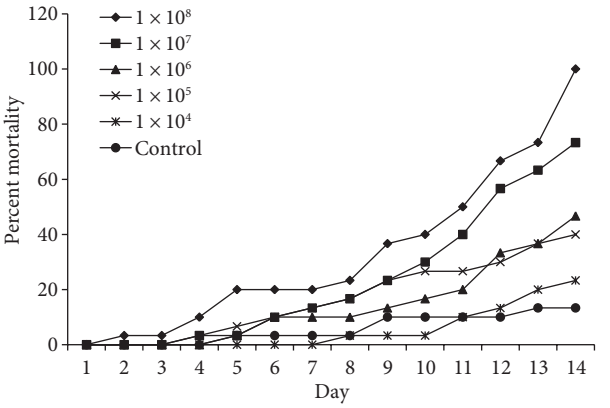

Figure 4. Cumulative mortality of *Corythucha ciliata* nymphs after application of 5 different doses of the spore concentration of *Beauveria bassiana* KTU – 24. Concentration unit is conidia mL^{-1}.

Table 2. Probit analysis parameters from the multiple-concentration bioassays performed with the *B. bassiana* isolate KTU – 24 against adult and nymph of *C. ciliata*.

Bioassay	Intercept	Slope ± SE[a]	LC_{50} (95% fiducial limits)	χ^{2b}	*df*
Adult	–3.802 ± 0.998	0.662 ± 0.168	5.51×10^5 (1.03×10^5 to 2.62×10^6)	1.811	3
Nymph	–3.211 ± 0.935	0.574 ± 0.158	3.96×10^5 (5.01×10^4 to 2.2×10^6)	2.064	3

[a]Slope of the concentration ± standard error response of adult and nymph of *C. ciliata* to *B. bassiana* isolate KTU – 24.
[b]Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$).

on wintering *C. ciliata* adults; however, no pathogenicity assay was conducted. Arzone and Ozino-Marletto (1984) compared 3 types of entomopathogenic fungi, including *B. bassiana* (Bals.) Vuill., *Verticillium lecanii* (Zimmerman), and *Paecilomyces farinosus* (Holmsk.), with respect to pathogenicity against *C. ciliata*, and they demonstrated that *B. bassiana* was the most virulent with 100% mortality. Arzone et al. (1986) also showed that *B. bassiana* was the most frequently isolated species from overwintering *C. ciliata* adults, i.e. 53% of isolations, but it was the least virulent. Ozino-Marletto and Menardo (1984) found that *B. bassiana* caused a 31% infection rate on *C. ciliata* adults. Ozino-Marletto and Arzone (1985) also showed that *B. bassiana* caused 100% mortality against *C. ciliata* at 30 °C and 100% relative humidity 6 days after treatment. Ozino and Zeppa (1988) performed infection tests with *B. bassiana*, *Verticillium lecanii*, and *Paecilomyces farinosus* on *C. ciliata* adults wintering on *Platanus* spp. of some city avenues and showed that *B. bassiana* was the most effective species with 25% mortality in the field experiment. Tarasco and Triggiani (2006) showed that *B. bassiana* caused up to 68% mortality against overwintering *C. ciliata* in the field. In our study, we also showed that a local isolate, *B. bassiana* KTU – 24, caused significant mortality against *C. ciliata* under controlled laboratory conditions. Although there are many studies about fungal biocontrol agents used for the control of *C. ciliata*, most have been conducted in Italy, and the investigation of local isolates against the target pest was warranted. In addition, entomopathogenic fungi are more likely to have ecological compatibility with pest species, due to their geographical locations and habitat types (Bidochka et al. 2001, 2002; Muro et al. 2003; Maurer et al. 1997). Therefore, it is suggested that native isolates have a more reduced risk of significant impact on nontarget organisms than exotic isolates. In this respect, *B. bassiana* isolate KTU – 24 appears to be a significant candidate organism for controlling *C. ciliata*, especially in Turkey, considering the origin of this isolate.

Moreover, *B. bassiana* KTU – 24 was also shown to be highly virulent against *Dendroctonus micans* (Kugel.) (Coleoptera:Curculionidae) and *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Thaumetopoeidae), which are important forest pests in Europe, including Turkey (Sevim et al. 2010a, 2010b). Based on these studies, there

is potential that *B. bassiana* KTU – 24 could be used as a biocontrol agent against a number of forest pests based on its high virulence on different forest pests in Turkey. Having a high virulence against different forest pests in the same region might be important advantage in terms of biological control to reduce application costs and labor, as well as the environmental impact. In this content, the isolate KTU – 24 could be further investigated for biopesticide development. We also considered the mycosis rate of the fungal isolates since sporulation is an important factor for dissemination and secondary recycling of the fungus in the field (Goettel et al. 2005). *B. bassiana* KTU – 24 caused the highest mycosis rate in both adults and nymphs of *C. ciliata*. This also supports the idea that isolate KTU – 24 could be further investigated as a possible microbial control agent against this pest.

Although there are a number studies on infection and use of *B. bassiana* against *C. ciliata*, no study about utilization of *M. anisopliae* against this pest is available in the literature. *M. anisopliae* is an important entomopathogenic fungus and is widely used for biocontrol of insect pests, and many commercial products are on the market or under development (Zimmermann 2007). This fungus has been shown to infect many important pest species including hemipteran insects (Marannino et al. 2006; Sahayaraj and Borgio 2009; Tiago et al. 2011). In this study, we also demonstrated that *M. anisopliae* isolates, especially KTU – 2, KTU – 27 and KTU – 60, might have potential for controlling *C. ciliata*.

I. fumosorosea (formerly known as *Paecilomyces fumosoroseus*) is a well-known entomopathogenic fungus with a worldwide distribution in temperate and tropical zones (Zimmermann 2008). This fungus is utilized for controlling many pest species worldwide (Mesquita et al. 1996; Gökçe and Er 2005; Zimmermann 2008; Avery et al. 2010). Chapin et al. (2006) showed that there was a decrease of 52% of the winter population of *C. ciliata* within 14 days after spraying of *P. fumosoroseus*. We also showed that *I. fumosorosea* KTU – 42 caused 63% and 50% mortality against *C. ciliata* adults and nymphs, respectively, indicating that this isolate might be good biological control agent against *C. ciliata*.

We did not determine a significant difference between adults and nymphs in terms of susceptibility to fungal isolates. In most entomopathogenic fungi, there is a differential virulence toward the life stages of insects, and not all stages in an insect's life cycle are equally susceptible to fungal infection (Goettel et al. 2005). In some cases, larvae are more susceptible than adults. For instance, *Dendroctonus micans* (Kugel.) (Coleoptera: Curculionidae) larvae are more susceptible than adults (Sevim et al. 2010b). In contrast, *Delia antique* (Meigen) (Diptera: Anthomyiidae) adults are more susceptible than larvae (Davidson and Chandler 2005). All these points suggest that there is no general rule regarding which development stages of an insect are more susceptible to fungal infection (Goettel et al. 2005).

The Eastern Black Sea Region of Turkey has favorable environmental conditions to use fungal entomopathogens in biocontrol programs because this region has a wet, humid climate and lower annual temperatures. The coastal parts of the region also receive the country's greatest amount of rainfall throughout the year (Ministry of Agriculture of Turkey 2007). Entomopathogenic fungi require moisture for sporulation and germination of conidia; some even need high humidity to initiate infection. In addition, rain plays an important role in transmission of entomopathogenic fungi (Goettel et al. 2005). In light of this information, it is obvious that entomopathogenic fungi can be the most appropriate candidate as a possible microbial agent to control *C. ciliata* in the Eastern Black Sea Region of Turkey. Therefore, the tested fungal isolates (especially KTU – 24) against *C. ciliata* in this study seem to be significant candidates in the Eastern Black Sea Region of Turkey.

In conclusion, we tested different entomopathogenic fungi against *C. ciliata* adults and nymphs under controlled laboratory conditions and demonstrated that the fungal isolates used in this study could be used as possible biocontrol agents against the pest. Among the tested fungi, it was found that *B. bassiana* KTU – 24 was the most promising according to its high mortality and mycosis values. Further studies should include determination of the effectiveness of this isolate in the field. Additionally, horizontal transmission studies between adults and nymphs are also warranted. Moreover, the side effects of the isolate KTU – 24 against natural enemies of *C. ciliata* should be also investigated.

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