Pathogenicity of Entomopathogenic Fungi to *Coccinella septempunctata* L. (Col.: Coccinellidae) and A Survey of Fungal Diseases of Coccinellids

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ABSTRACT: A survey to reveal naturally occurring entomopathogenic fungi on coccinellids in the East Mediterranean region of Turkey was conducted in 2004 and 2005. *Beauveria* and *Paecilomyces* species were found as pathogens of coccinellids. Fungal infection level in an overwintering site of coccinellids was significantly higher than those in the other locations. The pathogenicity of eight entomopathogenic fungi belonging to species (*P. farinosus, P. fumosoroseus, B. bassiana, Lecanicillium lecanii* and *Metarhizium anisopliae*) commonly considered as microbial control agents was investigated against *Coccinella septempunctata*. The experiment was conducted by treating the adults with an appropriate conidial suspension of 10^5 conidia ml^{-1} for a period of 10 seconds. The mortality was significantly higher in all the treatments comparing with the control unit. Overall mortality within 8 days after set of experiment varied between 27 to 51% and there were statistically significant differences amongst the effects of the tested fungi. The study showed that fungal diseases are natural mortality factors in coccinellid populations. Furthermore, it was demonstrated that not only naturally occurring fungal pathogens of coccinellids but also tested other entomopathogenic fungi isolated from non-coccinellid insect species were pathogenic to *C. semptempunctata*.
Key Words: Deuteromycotina, Hyphomycetes, insect pathology, microbial control, natural enemy

Entomopatojen Fungusların *Coccinella septempunctata* L. (Col.: Coccinellidae)'ya Patojenisiteleri ve Coccinellidlerin Fungal Hastalıkları Üzerine Bir Sürvey

ÖZET: Coccinellidlerin doğal hastalık etmeni olan entomopatojen fungusları belirlemek üzere 2004-2005 yıllarında Türkiye'nin Doğu Akdeniz bölgesinde bir sürvey yürütülmüştür. Coccinellidlerin patojenleri olarak *Beauveria* ve *Paecilomyces* türleri saptanmıştır. Bir kışlak bölgesindeki enfeksiyon düzeyi diğer lokasyonlardan önemli derecede yüksek bulunmuştur. Yaygın olarak mikrobiyal mücadele etmeni olarak ele alınan sekiz entomopatojen fungusun (*P. farinosus, P. fumosoroseus, B. bassiana, Lecanicillium lecanii* ve *Metarhizium anisopliae*) *Coccinella septempunctata*'ya patojeniteleri de test edilmiştir. Deneme erginlerin 10^5 konidispor ml^{-1} yoğunluğundaki uygun konidispor süspansiyonuna 10 saniye süreyle maruz bırakılması ile gerçekleştirilmiştir. Tüm uygulamalardaki ölüm oranları kontrol ünitesindekinden önemli derecede yüksek çıkmıştır. Sekiz gün içerisindeki ölüm oranı % 27 ile % 51 arasında gerçekleşmiş olup test edilen fungusların etkileri arasında istatistiksel olarak önemli farklılıklar bulunmuştur. Çalışma fungus hastalıklarının coccinellid popülasyonlarında doğal ölüm faktörleri olduğunu göstermektedir. Buna ilaveten, sonuçlar, sadece doğada görünen coccinellid patojeni fungusların değil aynı zamanda coccinellid olmayan diğer böcek türlerinden izole edilmiş olan ve çalışmada test edilen diğer entomopatojen fungusların da *C. septempunctata*'ya pataojenik olduğunu göstermektedir.
Anahtar Kelimeler: Deuteromycotina, Hyphomycetes, böcek patolojisi, mikrobiyal mücadele, doğal düşman

INTRODUCTION

Besides pest resistance related problems, ever-growing concerns about ecological and human health risks related with chemical insecticides contributed to increase interest in alternative pest management strategies to conventional insecticides. This includes consideration of entomopathogenic fungi as alternative control agents against many pest insects in biological control and integrated pest management programmes. Entomopathogenic fungi are used against pest insects and considerable research is directed to development of new microbial control agents (Inglis et al., 2001). These biopesticides are required to be compatible with natural enemies as well as presenting minimal risk to the environment and human health. Although some entomopathogenic fungi are known to have narrow host ranges, especially some of those belonging to Deuteromycotina have rather wide range of hosts from many insect orders, including natural enemies (Inglis et al., 2001).

Coccinellids are widely distributed and considered as important natural enemies of pest insects in biological control and integrated pest management programmes as they prey on a range of pests including aphids, scale insects, mealy bugs, spider mites and larvae of some species of Thysanoptera, Lepidoptera and Coleoptera (Hodek and Honék, 1996). Since it is not uncommon for coccinellids to coexist with targeted pest insects, some researches included or focused on pathogenicity tests against coccinellid species to evaluate the risk of using potential fungi as microbial control agents. In such studies, *Beauveria bassiana* (Bals.) Vuill. was generally found to be pathogenic to *Coccinella septempunctata* L. at various levels (Cagan

and Uhlik, 1999; Haseeb and Murad, 1997a,b; Manjula and Padmavathamma, 1996). Kiselek (1975), however, reported that a tested strain of *B. bassiana* was safe to *C. septempunctata*. *Beauveria* species were also encountered commonly on *C. septempunctata* (Cartwright et al., 1982; Ceryngier, 2000; Grigorov, 1983) along with infections by *Paecilomyces farinosus* (Holm ex S.F. Gray) Brown & Smith in a less extend (Ceryngier, 2000) in natural coccinellid populations. Furthermore, there are several reports on pathogenicity of entomopathogenic fungi to other coccinellid species (Ashouri et al., 2003; Bastidas, 1996; Ginsberg et al., 2002; James and Lighthart, 1994; Pavlyushin, 1996; Pell and Vandenberg, 2002; Peveling and Demba, 1997; Poprawski et al., 1998).

In this study, a survey was conducted to reveal naturally occurring entomopathogenic fungi on coccinellids in the East Mediterranean region of Turkey in 2004-2005, and the pathogenicity of several entomopathogenic fungus species, commonly considered as microbial control agents, was also tested against *Coccinella septempunctata*.

MATERIALS and METHODS

Isolation of Natually Occurring Fungal Pathogens of Coccinellids

Adult coccinellids were collected from mainly cultivated fields in Kahramanmaraş, Osmaniye, Mersin and Adana in Turkey in 2004-2005. Insects suspected to be infected were placed in small vials individually in field. They were surface sterilized by dipping into ethanol and sodium hypochlorite solution and sterile distilled water (Lacey and Brooks, 1997) upon their arrival to laboratory prior to being placed in humidity chambers. Additional collected insects were placed in large plastic containers and returned into laboratory. Either hand collection or sweeping methods were employed depending on the plantation. All collected insects were kept in a climate controlled room (20±2°C, 60±5% RH and 8/16 photoperiod). Coccinellids were fed by providing aphids and honey solution and checked daily to detect any symptoms of fungal diseases for two weeks. Those exhibiting any symptoms were also surface sterilized and taken into humidity chambers as described above unless fungal growth had already started. Sealed petri dishes lined with wet blotting paper were employed as humidity chambers.

Once the outgrowth of any fungi was detected on insects, the seal of the humidity chambers were removed to encourage sporulation. Fungi were further studied after the sporulation occurred. First, the cadavers invaded by common saprophytic fungi (*Penicillium* spp., *Mucorales* spp., *Aspergillus* spp., etc.) were eliminated from further studies. Fungi on the other cadavers were isolated and cultured on potato dextrose agar (PDA) in petri dishes in an incubator at 24±2°C in darkness. The sporulation structures of the fungi were examined under a phase-contrast light microscope for identification. Yates correction for continuity (Zar, 1996) was used to calculate chi-square values to perform pairwise chi-square tests in order to assess the differences in prevalence between collection sites. Uludaz is an overwintering site in Kahramanmaraş, therefore data from this site was treated separately.

Organisms

Paecilomyces farinosus, *P. fumosoroseus* (Wize) Brown & Smith, *Lecanicillium lecanii* (Zimm.) Zare & W. Gams isolates, *Beauveria bassiana*, isolate no: 1151, and *Metarhizium anisopliae* (Metschnikoff) Sorokin, isolate no: 3329, were provided by Dr R. HUMBER, *M. anisopliae*, isolate no: FI 23, was provided by R. MILNER and *B. bassiana*, isolate no: 215, was provided by Dr. M. S. Dayer. The fungi were cultured on PDA in an incubator at 24±2°C in darkness. Adults of *C. septempunctata* were brought into laboratory from one location in Kahramanmaraş and the insect cultures were kept in a climate controlled room (20±2°C, 60±5% RH and 8/16 photoperiod) until they are used. Insects with abnormalities were removed, and during the checks before further use, disease symptoms were not detected on the remaining insects. Insects for the test were chosen amongst these insects without any bias.

Pathogenicity Test of The Fungi to *C. septempunctata*

Once the fungi completely sporulated, four weeks after inoculation, conidia in each petri dish were collected in 5 ml of 0.1% sterile Tween 80. The conidial suspensions were passed through two layers of sterile muslin to remove any agar pieces and hyphae from the conidial suspensions. Their concentrations were determined by three counts of conidia on Thoma counting slides under a phase-contrast microscope at 40X magnification. All the conidial suspensions were diluted so that the same concentration of 10^5 conidia ml^{-1} was reached for all the suspensions. These suspensions were kept at +4°C overnight before using for the pathogenicity tests.

For each fungus, 20 adult coccinellids were dipped together into an appropriate conidial suspension for 10 seconds. Insects treated with 0.1% sterile Tween 80 composed the control unit of the experiment. They were placed in a petri dish lined with wet blotting paper for 24 hours before moving to the insect culturing conditions. Thereafter, the insects were checked daily and mortality was recorded. Dead insects were transferred into humidity chambers to monitor any fungal out-growth as previously described for detection of naturally occurring fungal diseases of coccinellids. The experiment was repeated three times. The overall percentage mortality within eight days after treatment was subjected to ANOVA following arcsine transformation (Zar, 1996) by using Minitab statistics software. The differences amongst the treatments were

determined by subjecting the data to Tukey multiple comparisons test.

Table 1. Mortality of collected coccinellid adults and the incidence of insects that died due to diseases caused by entomopathogenic fungi

Place of collection	Number of examined insects	Incidence of mortality	Death followed by mycosis (%)*
Kahramanmaraş	2780	392	2.66 a
Adana	70	4	0.00
Osmaniye	114	23	0.88 a
Mersin	270	28	0.00
Uludaz	2207	172	3.08 b
Overall	5441	619	2.63

*Different letters indicate statistically significant difference according to Pairwise chi-square tests with Yates correction for continuity, $P<0.05$

RESULTS

Naturally Occurring Fungal Diseases of Coccinellids

Majority of the collected coccinellid adults were identified as *C. septempunctata*. Other common species were *Adonia variegata* (Goeze) and *C. undecimpunctata* (L.). The insects died and supported the growth of entomopathogenic fungi under laboratory conditions were all found to be *C. septempunctata*. Although fungal growth was noticed on most of the cadavers, fungi grew were generally one of common saprophytic fungi such as *Penicillium* spp., Mucorales., *Aspergillus* spp. Those died due to an entomopathogenic fungus were found to be killed as a result of mycosis caused mostly by *Beauveria* sp. and rarely by *Paecilomyces* sp. The identification was made according to Humber (Humber, 1997). The incidence was the highest in Uludaz, an overwintering site of *C. septempunctata* in Kahramanmaraş while none of the insects collected from Adana and Mersin, two cities by the Mediterranean Sea, developed mycosis due to any entomopathogenic fungi (Table 1). According to pairwise chi-square tests, the prevalence in Uludaz was significantly higher ($P<0.001$) than those in Kahramanmaraş ($X^2=26.88$) and Osmaniye ($X^2= 11.38$) while there was not a significant difference between the incidences in these two cities ($X^2=3.74$, $P<0.05$).

Effects of Tested Fungi on *C. septempunctata*

Screening test of eight entomopathogenic fungal isolates against *C. septempunctata* adults resulted in various mortality levels ranging from 27.41 to 50.93% depending on fungus species and isolates. ANOVA test revealed statistically significant differences amongst the treatments ($F=40.13$; $P<0.05$; $sd=8.26$). All the fungi applied gave a significantly higher mortality than the control unit (Table 2) and there was not any insect on which an entomopathogenic fungus grew in the control unit. The data shows intra-specific differences between the isolates of both *P. farinosus* and *B. bassiana* as well as inter-specific differences amongst the tested fungus species (Table 2). High pathogenicity was not only expressed by naturally occurring pathogen species (*B. bassiana* and *P. farinosus*) reported on *C. septempunctata*, but *L. lecanii* and *M. anisopliae* were also equally pathogenic. However, all the fungal isolates were poor in sporulation on the host insect (Table 2).

Table 2. Pathogenicity of tested entomopathogenic fungi to adults of *Coccinella septempunctata* in controlled conditions.

Entomopathogenic fungus	Original host species	Mortality * (±s.e.) (%)	Sporulation occurred**
Paecilomyces farinosus (ARSEF-4045)	*Hapalus sp.*	27.41 ± 3.88 c	4
Beauveria bassiana (ARSEF-1151)	*Delia radicum*	48.89 ± 1.11 ad	3
Lecanicillium lecanii (ARSEF-5132)	*D. radicum*	50.93 ± 3.28 a	5
P. fumosoroseus (ARSEF-3458)	*Trialeurodes vaporariorum*	39.49 ± 0.51 bde	2
Metarhizium anisopliae (ARSEF-3329)	*Popillia japonica*	47.61 ± 1.32 ade	7
P. farinosus (ARSEF-4010)	*Agrotis segetum*	49.02 ± 0.98 ad	4
M. anisopliae (CSIRO-F1-23)	*Aeneolamia albofasciatat*	38.33 ± 0.83 be	11
B. bassiana (HRI-215)	Isolated from "Boverin"by E.A. Grula	36.94 ± 1.94 bc	2
Control		13.47 ± 1.20 f	0

*Different letters indicate a statistically significant difference according to Tukey multiple comparison test, $P<0.05$. Values are average of three replicates (n=20).

**Number of insects in three replicates

DISCUSSION and CONCLUSION

Although the infection level was not very high, this study shows that fungal diseases (*Beauveria* spp. and *Paecilomyces* spp.) are natural mortality factors in coccinellid populations in the region covered. Similar natural fungal infections were also reported previously in other countries (Cartwright et al., 1982; Ceryngier, 2000; Grigorov, 1977, 1983; Iperti, 1986; Lipa et al., 1975). As it was the case in the results presented here, a common finding is that fungal diseases of coccinellids are caused mostly by *Beauveria* species (Cartwright et al., 1982; Ceryngier, 2000; Ghazavi et al., 2005; Grigorov, 1977, 1983; Iperti, 1986; Lipa et al., 1975) and sometimes by *Paecilomyces* species (Ceryngier, 2000). Furthermore, this study revealed that prevalence of fungal infections was significantly higher in an overwintering site (Uludaz) than those in the other locations. Ceryngier (Ceryngier, 2000) also found the incidence to be higher in an owervintering site in Poland. High population level of coccinellids in overwintering sites with higher humidity level and relatively lower temperature could favour the entomopathogenic fungi for survival and spread.

The fungi tested against *C. septempunctata* adults in this study caused significantly higher mortality comparing with the control unit. Therefore, all these fungal isolates can be considered pathogenic to *C. septempunctata* at various levels. *B. bassiana* was previously reported to be pathogenic to this predator species in several studies (Cagan and Uhlik, 1999; Haseeb and Murad, 1997a,b; Manjula and Padmavathamma, 1996). The present study also demonstrated the pathogenic feature of *P. farinosus*, *P. fumosoroseus*, *L. lecanii* and *M. anisopliae* against *C. septempunctata* and their effects on the host was not less than that of the *Beauveria* isolates tested here. Other coccinellid species were previously found to be susceptible to these fungal species. *Hippadomia convergens* Guerin was found to be susceptible to *M. anisopliae* (Ginsberg et al, 2002; James and Lighthart, 1994); *Cycloneda limbifer* Casey to *P. fumosoroseus* (Pavlyushin, 1996); *Adonia variegata* to *L. lecanii* (Ashouri et al., 2003); and *Serangium parcesetosum* Sicard to *P. fumosoroseus* (Poprawski et al., 1998). Even though our findings and previous researches indicate that coccinellid predators could be at risk due to use of fungi as microbial control agents, some conflicting results by other studies showed that entomopathogenic fungal strains non-pathogenic to these natural enemies can also be found (Bastidas, 1996; Kiselek, 1975; Pell and Vandenberg, 2002; Peveling and Demba, 1997).

It is worth noticing that despite the fact that *C. septempunctata* can be infected by several entomopathogenic fungal species, only *B. bassiana*, *B. brogniartii* (Saccardo) Petch and *P. farinosus* have been encountered in natural populations both in this and previous studies. This may be explained by differences amongst entomopathogenic fungi in terms of their success to develop an actual mycosis on each host species. The fungi tested in this study developed weak mycosis on the insect cadavers.

This study shows that coccinellids can suffer from fungal diseases in nature, and fungi from species that were not encountered in nature can also infect and kill them at various levels. The experiment was carried out in a worst case scenario and reflects specially laboratory host range, therefore, further tests under conditions that mimic natural conditions are needed for better understanding of the risk of entomopathogenic fungi to *C. septempunctata*. Both this study and previous researches also show that pathogenicity of entomopathogenic fungi to coccinellids depends on fungal isolate and host species as well as other factors such as climate and application procedure. Therefore, in the selection of an entomopathogenic fungal isolate as a microbial agent against pest insects, tests revealing the effects of considered pathogens against natural enemies should be conducted for each isolate to each related natural enemy species.

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