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# Effects of some entomopathogenic fungi on Citrus Mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)

## ABSTRACT

Citrus Mealybug *Planococcus citri* is an economically important pest species causing harm on citrus and some other plants. Biological control of the pest is gaining importance because of the problems caused by chemical control. In this study, effects of *Beauveria bassiana, Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* against second instar larvae of mealybug were investigated. Healthy potato tubers were dipped into spore suspensions of the entomopathogens with $10^8$ conidia/ml concentration and then infested with mealybugs. Potatoes were then kept in a climatic room with 25±1°C temperature and 60±5% humidity. Alive and dead mealybug individuals were determined 3, 5 and 7 days after applications and mortality rates were calculated. As a result, three entomopathogens caused different rates of mortality on the pest. *B. bassiana* isolate showed the highest effect and killed all pest individuals 5 days after the application. Mortality rates caused by *P. fumosoroseus* and *P. lilacinus* were lower in the first two observations, while they showed 96% and 82% mortality on the seventh day, respectively.

Key words: *Beauveria bassiana, Paecilomyces fumosoroseus*, *Paecilomyces lilacinus*, *Planococcus citri*

## INTRODUCTION

The Citrus Mealybug *Planococcus citri* (Risso), Hemiptera: Pseudococcidae, is an economically important polyphagous pest species causing harm mainly on citrus and some other plants such as; olives, vineyards, pomegranate, banana, melon, watermelon and ornamentals in Turkey (Karacaoğlu and Yarpuzlu, 2007). It causes premature leaf drop, dieback and also can cause death of plant if not controlled (Polat *et al.*, 2008). This pest not only directly damage plants by sucking plant sap, but also cause indirect damage transmitting some viral plant diseases as a virus vector (Jones and Lockhart, 1993; Garnsey *et al.*, 1998; Cid *et al.*, 2010). Causing the formation of soothy molds by secreting honeydew is another indirect effect of the pest (Lodos, 1986). Chemical control is often ineffective because of the wax layer on the insect, its protected localization and disordered distribution and also because of the development of resistant populations. This is why alternative

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methods such as biological control became important in the management of this pest (Flaherty *et al.*, 1982; Mani *et al.*, 2011; Correa *et al.*, 2015). Previous studies showed that entomopathogenic fungi can successfully be used against citrus mealybug. In a laboratory study, pathogenicity of oil and water based suspensions of *Metarhizium anisopliae* (Metchnikoff) and *Beauveria bassiana* (Bals. -Criv. ) Vuill. was investigated and virulent isolates were found (Cannard *et al.*, 2002). In another study, mortality rates between 45-91% were obtained by the *in vitro* application of a single dose ($10^7$ spores/ml) of *Verticillium lecanii* (Zimmerman), *B. bassiana*, *B. brongniartii* (Saccardo), and *M. anisopliae* against *P. citri* where the first entomopathogen showed the highest efficiency. In the same study, a highest dose ($10^9$ spores/mL) of *V. lecanii* caused 95% mortality of the pest (Saranya, 2008). In Turkey, it was found that $10^8$ conidia/ml concentration of *Paecilomyces farinosus* (Holmsk. ) caused about 85% mortality in adult females of citrus mealybug, and that mortality rates decreased with the decreasing levels of humidity and inoculum density (Demirci *et al.*, 2011). In a recent *in vitro* study, $10^7$ spores/ml concentration of *B. bassiana* and *M. anisopliae* showed 67. 5 and 64% mortality on citrus mealybug crawlers respectively and mentioned as potential biocontrol agents of the pest (Fitz-Gerald *et al.*, 2016).

The aim of this study was to investigate the effects of *B. bassiana, P. fumosoroseus* (Wize) and *P. lilacinus* (Thom) Samson isolates which were previously isolated from *Hyphantria cunea* (Drury) (Lepidoptera: Erebidae) pupae and caused varying degrees of mortality on this pest (Sullivan *et al.*, 2011), against mealybug under laboratory conditions.

## MATERIALS AND METHODS

### Mass rearing of citrus mealybug

*P. citri* individuals used in the study were obtained from the culture reared on pumpkins (fig. 1) in the Biological Control Laboratory of Plant Protection Department (Suleyman Demirel University, Faculty of Agriculture, Isparta, Turkey). Second instar larvae of the citrus mealybug were used in the study.

### Entomopathogen isolates and preparation of the spore suspension

*B. bassiana, P. fumosoroseus* and *Paecilomyces lilacinus* isolates previously isolated from *H. cunea* overwintering pupae and kept in the culture collection of the Mycology Laboratory of Suleyman Demirel University, Faculty of Agriculture, Department of Plant Protection were used as entomopathogens in the study. Fungi

Fig. 1 - *Planococcus citri* individuals on an infested pumpkin.

were subcultured on PDA plates and incubated at 25 °C in the dark for 2-3 weeks. Spores were then transferred to a sterile glass bottle by scraping with a sterile scalpel and by washing with sterile distilled water containing 0. 02% Tween 20, through two layers of cheesecloth, in order to separate mycelia and the spores. Concentration of the spore suspensions of the entomopathogens was adjusted to $10^8$ conidia/ml by using a haemocytometer.

### Bioassay

Healthy and clean potato tubers were dipped into spore suspensions for five seconds, blotted dry on sterile blotter papers and then each tuber was infested with 10 second instar larvae of the mealybug. Potatoes were then transferred to 10x10x5 cm plastic boxes and kept in a climatic room with 25±1°C temperature and 60±5% humidity. Trial was performed in a completely randomized design with 5 replicates. Alive and dead mealybug individuals were counted 3, 5 and 7 days after applications. Dead individuals were transferred to PDA plates and checked for mycosis by the infested fungi. Mean numbers of alive individuals and mortality rates were subjected to analyses of variance and means were compared by Tukey's Honestly Significant Difference Test (P=0. 05).

## RESULTS

In the first observation made 3 days after the applications, all entomopathogens started to affect the pest individuals and statistically arranged in different groups from the control group where all individuals were alive (tab. 1). It was determined that *B. bassiana* affected the pest more quickly than the other two entomopathogens and killed all larvae 5 days after inoculation (fig. 2).

The lowest mortality rate was obtained by *P. fumosoroseus* in the 3rd day, while the entomopathogen showed better effect in the further observations and arranged in the same group with *B. bassiana* in the 7th day. Although *P. lilacinus* and *P. fumosoroseus* could not kill all *P. citri* larvae in the experiment, they caused 82% and 96% mortality rates on the seventh day, respectively. Inoculated entomopathogens were reisolated from the dead mealybugs, so fulfilling Koch's postulates.

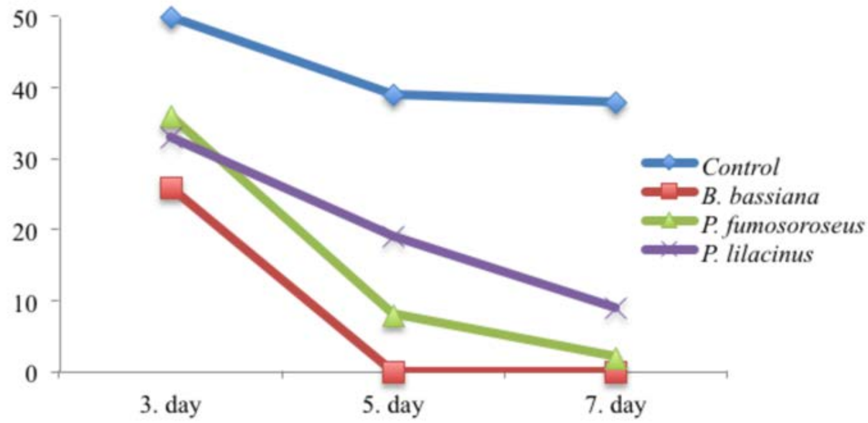


Fig. 2 - Numbers of alive *Planococcous citri* individuals 3, 5 and 7 days after inoculation with entomopathogens.

Tab. 1 - Mortality rates (%) of *P. citri* individuals on potato tubers inoculated with entomopathogens.

| **Fungi** | **Days after inoculation** | | |
|---|---|---|---|
| | 3rd day | 5th day | 7th day |
| *B. bassiana* | 48 ± 4. 89 a | 100 ± 0. 00 a | 100 ± 0. 00 a |
| *P. lilacinus* | 34 ± 2. 44 ab | 62 ± 3. 74 c | 82 ± 3. 74 b |
| *P. fumosoroseus* | 28 ± 5. 83 b | 84 ± 2. 44 b | 96 ± 2. 44 a |
| Control | 0 ± 0. 00 c | 2 ± 2. 00 d | 4 ± 2. 44 c |

## DISCUSSION

In this research performed to investigate the effects of three entomopathogens; *B. bassiana*, *P. fumosoroseus* and *P. lilacinus*, the former entomopathogen showed the best activity and killed all pest individuals 5 days after the transformation of the pest onto the potato tubers infested with spore suspensions of the fungi. *Paecilomyces* species failed to kill all pest individuals during the experiment, but they still can be mentioned as potential biocontrol agents in terms of the high mortality rates caused by them. In a similar study, *B. bassiana* isolates caused about 68% and 64% mortality when spore suspension with $10^7$ concentration was applied onto the pest crawlers, but mortality rates of the adults subjected to the same spore concentration were lower (Fitz-Gerald *et al.*, 2016). Difference between the mortality rates obtained in this study and our study may because of the virulence differences among the fungal isolates, the stages of the pest and the experimental conditions. In another study, it was found that *P. farinosus* showed 84% mortality on the same stage of the pest when used at the same concentration under 95% relative humidity and it was mentioned that humidity is an important factor affecting the efficiency of the entomopathogen (Demirci *et al.*, 2011). So, it can be mentioned that our isolates may be more effective under more humid conditions. Performance of the entomopathogenic isolates used in this study should better be investigated under natural conditions.

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