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# Potential of single and combined releases of *Eretmocerus mundus* and *Macrolophus melanotoma* to suppress *Bemisia tabaci* in protected eggplant

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ABSTRACT

The parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and the predator *Macrolophus melanotoma* (Costa) (Hemiptera: Miridae) are two important natural enemies of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) which are distributed throughout the Mediterranean region. Both natural enemies are used in the biological control of *B. tabaci*. In this study, the success of single and combined release of *Er. mundus* and *M. melanotoma* against *B. tabaci* on eggplant was determined in 3 × 3 × 2.5 m net cages established in a greenhouse during the period of 2009 and 2010. Four different experiments were conducted; *Er. mundus* single release, *M. melanotoma* single release, *Er. mundus* + *M. melanotoma* combined release, and *B. tabaci* single release (control). For the evaluation of population development of whitefly and the parasitoid, leaf samples were taken at five day intervals. In addition, numbers of the predatory insects were calculated by using visual control method on whole parts of 15 plants in different treatments. Results of this study showed that the number of whiteflies was the highest in the control treatment, followed by the *M. melanotoma* (single), *Er. mundus* (single) and *Er. mundus* + *M. melanotoma* (combined) treatments, in both years. Low *B. tabaci* populations were observed in combined release treatments and the weekly mean density of immature whiteflies never exceeded 3.99 per $cm^2$ leaf area. *M. melanotoma* was not successful against *B. tabaci* when released alone. However, it does contribute to the success of biological control of *B. tabaci* when released with *Er. mundus*. In light of these results, we suggest the combined release of *Er. mundus* and *M. melanotoma* for effective control of whitefly in greenhouse grown eggplants.

## 1. Introduction

The sweetpotato whitefy *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a worldwide agricultural pest, attacking a wide range of hosts, including eggplant, *Solanum melongena* L. (Solanales: Solanaceae). Currently, it is considered as a species complex consisting of at least 40 cryptic species that can be identified by molecular methods (Elfekih et al., 2018). *Bemisia tabaci* is causing economic losses by direct feeding as well as indirectly by acting as a vector of several viral diseases (Jones, 2003; Dombrovsky et al., 2013; Abd-Rabou and Simmons, 2015).

Insecticides are widely used by eggplant growers to suppress *B. tabaci* populations (Sujayanand et al., 2015; Ulubilir and Yabas, 1996; Abd-Rabou and Simmons, 2015). The application of biological control strategies against pests occurring in greenhouse are also used successfully. These treatments provide long-term pest control and offer several benefits including the absence of pest resistance and of pesticide residues on the marketable products (van Lenteren, 2006; Kazak et al., 2015). For this reason, development of biological control programs is an attractive solution to suppress whitefly in greenhouse grown eggplants.

Several natural enemies including parasitoids and predators have been commercialized by many companies for augmentative biological control of *B. tabaci* (Gerling et al., 2001; Alomar et al., 2006; Arno et al., 2010). However, compared with the commercial populations, local populations of these natural enemies may be more effective due to adaptation to their environment. The parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), as well as the predators *Macrolophus melanotoma* (Wagner) and *M. pygmaeus* (Rambur) (Hemiptera: Miridae), are recognized as important indigenous natural enemies of *B. tabaci* that can be found across all parts of the Mediterranean region and Turkey (Alomar et al., 1994; Gerling et al., 1998; Ulusoy, 1999; Castane et al., 2004; Urbaneja and Stansly, 2004; Perdikis et al., 2007; Karut et al., 2012, 2016). *Eretmocerus mundus* is the most abundant parasitoid recovered spontaneously from *B. tabaci* in greenhouses (Lopez and Andorno, 2009; Arno et al., 2010; Karut et al., 2012). The efficacy of

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this parasitoid has been mostly studied on pepper and tomatoes, with limited study on eggplant (Stansly et al., 2004,2005a,b; Urbaneja et al., 2007; Karut et al., 2016). Further investigations are warranted, as success of *Er. mundus* is not always stable and varies depending on the host plant species (Urbaneja et al., 2007; Malik and Karut, 2012).

Moreover, *M. pygmaeus* is known to be associated with solanaceous plants such as *S. lycopersicum* (tomato) and *S. nigrum* L. (Solanaceae), while *M. melanotoma* occurs on the non-cultivated plant *Dittrichia viscosa* L. (W. Greuter) (Asteraceae) throughout the year, reaching the highest numbers in the middle of the summer months (Lykouressis et al., 2012; Evangelou et al., 2013). Although efficacy of *M. pygmaeus* is well documented on various crops and various prey species, the efficacy of *M. melanotoma* has been rarely addressed (Montserrat et al., 2000; Enkegaard et al., 2001; Perdikis and Lykouressis, 2002; Montserrat et al., 2004; Lykouressis et al., 2007, 2012). Furthermore, experiments investigating the relative preference of *M. melanotoma* when given access only to solanaceous plants revealed that it prefers eggplant at higher rates than pepper and tomato (Lykouressis et al., 2012).

In suppression of pest populations, combined release strategy provides more successful results than separate use of natural enemies in pest management, including whiteflies (Heinz and Nelson, 1996; Harvey and Eubanks, 2005; Chailleux et al., 2013; Berger et al., 2017; Rocca and Messelink, 2017). Heinz and Nelson (1996) showed that releases of *Delphastus pusillus* LeConte (Coleoptera: Coccinellidae) in combination with one or both of the parasitoids (*Encarsia formosa* and *En. pergandiella*) provided the greatest levels of whitefly suppression in poinsettias. Although predatory mirids and aphelinid parasitoids are well known biological control agents of *B. tabaci*, few studies have explored combined release of these natural enemies (Gabarra et al., 2006; Moreno-Ripoll et al., 2014). Combined releases of *Er. mundus* with *Macrolophus* species [e.g *M. pygmaeus*, *M. melanotoma* (= *caliginosus*) and *M. costalis*] may provide more successful results than separate employment of these two natural enemies in suppression of whitefly populations (Gerling et al., 2001). This suppression has been demonstrated through a series of studies in tomato, however, there remains a dearth of research on the host eggplant (Gabarra et al., 2006). Therefore, the aim of this study was to determine the potential of single and combined releases of two indigenous natural enemies *Er. mundus* and *M. melanotoma* to suppress *B. tabaci* populations in protected eggplant.

## 2. Material and methods

### 2.1. Prey, predator, parasitoid identification and rearing

The initial population of the prey *B. tabaci* was collected from a commercial cotton field in Adana, Turkey. The stock culture was established on various host plants namely *S. lycopersicum* (tomato), *S. melongena* (eggplant) and *Gossypium hirsitum* L. (cotton). According to *Vsp*I-based mtCOI polymerase chain reaction–random length polymorphism (PCR-RFLP) molecular analyses, the species of the *B. tabaci* was determined as MEAM1 (Middle East Asia Minor 1) (B biotype) (Khasdan et al., 2005).

Prior studies have reported host plant specification between *M. melanotoma* and *M. pygmeous* species (Martinez-Cascales et al., 2006; Lykouressis et al., 2012; Castane et al., 2013; Evangelou et al., 2013). The species found on *Dittrichia viscosa* (Asteraceae) is *M. melanotoma*, and the species preferring solanaceous plants is *M. pypmaeus* (Castane et al., 2013; Evangelou et al., 2013). The initial population of predator used in this study was collected from *D. viscosa* plants in Mersin, Turkey. The predator was maintained on whitefly-infested host plants, which were transferred from prey rearing to the net cages 60 × 45 × 30 cm in size. After two generations 30 individuals were preserved in 96% ethyl alcohol for further molecular analyses. Molecular identification was carried out using the specific primers (Mp1F/Mp4R and Mm1F/Mm3R) suggested by Castane et al. (2013), and the results confirmed that the species used in this study is *M. melanotoma*.

Parasitoids were obtained from cotton leaves infested with *B. tabaci* in an experimental farm field of Çukurova University, Adana. The parasitoids were identified as *Er. mundus* using morphological characters such as antenna, colorization of the first two abdomen segments and ovipositor features as indicated by Sharaf (1982). After identification, parasitoids were released in similar cages (in size) as described above in which various species of host plants infested with 2nd instar larvae of *B. tabaci* were located (Karut, 2007). All insect rearing were performed at 25 °C, 60–70% RH and 16:8h Light: Dark photoperiod regimen.

### 2.2. Experimental design

Experiments were conducted over two growing seasons in spring of 2009 and 2010 at a greenhouse located at Çukurova University, Adana, Turkey. The experiments were established using net cages 3 × 3 × 2.5 m in size (1 mm square mesh). Four different treatments were established, namely: "Treatment 1" *Er. mundus* single release, "Treatment 2" *M. melanotoma* single release, "Treatment 3" *Er. mundus* + *M. melanotoma* combined release, and "Treatment 4" *B. tabaci* single release (control). The experiments were arranged in a complete randomized design in total of twelve cages (three cages for each treatment). Approximately 30 eggplant seedlings (*Solanum melongena* cv Anamur) obtained from a nursery were planted in each cage on March 9, 2009 and April 13, 2010. In all treatments, *B. tabaci* were released when seedlings had four leaves (15 days later). In 2009, 5 individual whiteflies were released per plant. In the second year (2010), the initial density of the whitefly was increased threefold (15 individuals released), to assess the success of parasitoids against a higher density of pests. In order to obtain suitable prey stage (2nd instars larvae) for the parasitoids (Karut, 2007), *Er. mundus* releases were conducted 10 days after prey release. Concurrently, the predator *M. melanotoma* was also transferred to the cages. For the parasitoid treatments, 6 and 15 individual *Er. mundus* were released, per plant, in 2009 and 2010, respectively (release ratio was 1 host: 1 parasitoid for both years). The initial host: parasitoid ratios used in this study were determined based on the release rates used in tomatoes and peppers given by Gabarra et al., 2006 and Lopez and Andorno, 2009. In both years, one gravid female *M. melanotoma* was released per plant (release ratios were 1:5 and 1:15 for first and second years). Natural enemy releases were made once every two years. All insects were obtained from the stock colony using a mouth aspirator and transferred in Eppendorf tubes. When transferring *M. melanotoma* and *Er. mundus*, a small piece of gauze was placed in the tube to protect insects from physical damage.

### 2.3. Sampling

Sampling was started fifteen days after the last insect release. On each sampling date, a total of 30 leaves (10 leaves from each cage) per treatment were taken randomly from the same level (middle) of the different seedlings. The leaves were placed in paper bags and kept in an icebox until transferred to the laboratory. On each leaf, five different subsamples (each was 4 cm square in size) were constructed using a heavy paper template. All immature stages (except egg) of *B. tabaci* and *Er. mundus* pupae were counted with the aid of a 10× binocular microscope.

Additionally, at each sampling date, the abaxial surface of 30 leaves per cage (from the upper level of the seedlings) were examined non-destructively, and adults of *B. tabaci* were calculated by the visual control method (without the use of a microscope). During the sampling period nymphs and adults of *M. melanotoma* were also calculated by the visual control method. Towards this aim, from each cage, all parts of 15 randomly selected seedlings were checked and numbers of *M. melanotoma* were recorded.