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Compatibility of endophytic fungal entomopathogens with plant extracts for the management of sweetpotato whitefly *Bemesia tabaci* Gennadius (Homoptera: Aleyrodidae)

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GRAPHICAL ABSTRACT

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ABSTRACT

The effects of individual and combined application of endophytic fungal entomopathogens (*Beauveria bassiana* strain NATURALIS and *Metarhizium brunneum* strain BIPESCO5) and aqueous extracts of two medicinal plants (*Calotropis procera* and *Inula viscosa*) on the survival and development of the sweetpotato whitefly (*Bemisia tabaci*) were investigated for the first time. *Brassica oleracea* was inoculated through foliar spray of plants with the conidial suspension of fungal strains, and the endophytic colonization of different plant parts (leaf, stem, and root) was confirmed 7 days post-inoculation (7 dpi); when *B. tabaci* adults were introduced into plants and prior to plant extract application. 48 h later, whitefly adults were removed and the egg bearing leaves of respective treatments were sprayed with aqueous plant extracts. Although all treatments had a significant negative effect on the survival of different *B. tabaci* developmental stages compared to control, an increase in percentage mortality among all developmental stages was consistently observed when combined applications of endophytic entomopathogenic fungi and aqueous plant extracts were used; irrespective of fungal strain or plant extract. However, the increase was not always additive. Combined application of endophytic entomopathogenic fungi and plant extracts had an additive effect on mortality of nearly all whitefly developmental stages when endophytic *B. bassiana* was applied with *C. procera* extract. On the other hand, when endophytic *M. brunneum* was applied with either plant extract, the combined effects were always significantly higher than effects achieved by individual treatments; but occasionally additive. Similarly, whitefly development was significantly delayed when individual and combined applications of endophytic fungal entomopathogens and plant extracts were used; but the delay was most significant in response to combined applications. Our results provide the first report for the compatible use of fungal entomopathogens, applied as endophytes, with aqueous plant extracts for the management of insect pests; particularly *B. tabaci*.

1. Introduction

The sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) comprises a complex of biotypes or, as more recently suggested, a complex of at least 24 morphologically indistinguishable species (De Barro et al., 2011). Among these, *B. tabaci* biotype B (Middle East–Asia Minor 1), also known as the silverleaf whitefly *Bemisia argentifolii* (Bellows and Perring) (Bedford et al., 1993; Bellows

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et al., 1994), is particularly considered as one of the most important agricultural pests that has caused tremendous economic losses of vegetables, ornamentals, and field crops in many countries worldwide, including Jordan (De Barro et al., 2011). Losses occur when this polyphagous insect attacks and damages crops in diverse ways, including direct feeding, induction of plant physiological disorders, accumulation of honeydew or sooty mold, and most importantly, transmission of plant viruses (Oliveira et al., 2001). Whitefly management programs have been largely dependent on chemical control (Horowitz and Ishaaya, 1996). However, repeated and intensive insecticide applications have resulted in the rapid development of resistant *B. tabaci* populations to a number of conventional and novel insecticides, in addition to ecological disturbances and higher costs to growers (Horowitz et al., 2004; Basit et al., 2013). To combat insecticide resistance in *B. tabaci* and achieve effective pest control, management strategies should couple chemical control with the use of other Integrated Pest Management (IPM) tactics such as biological control (Palumbo et al., 2001).

The use of biological control agents, for example entomopathogenic fungi, has been considered as one of the alternative approaches to control *B. tabaci* (Faria and Wraight, 2001). Microbial biological control by fungal entomopathogens has several advantages over chemical control, such as the low likelihood of resistance development (Gao et al., 2017), safety to non-target organisms (Roy and Pell, 2000), decreased negative impacts on human health (Baltazar et al., 2014), and lower risks of environmental contamination (Goettel and Johnson, 1994). In spite of the development and registration of an increasing number of entomopathogenic fungi-based products for whitefly biological control under both greenhouse and field conditions, several factors continue to limit achievement of the full potential of these fungi as effective biocontrol agents against this pest worldwide. These factors mainly include dependence on favorable environmental conditions, slow action and limited shelf-life, potential incompatibility with chemical fungicides applied for disease control, and difficulty in targeting the pest due to preference of whiteflies for the undersides of leaves (Faria and Wraight, 2001). Research efforts have been initiated to identify different possible means to overcome such constraints (Wraight and Carruthers, 1999).

Delivery of entomopathogenic fungi as endophytes (microorganisms asymptomatically colonizing inner plant tissues for some or all of their lifecycle; Wilson, 1995) might offer a promising opportunity to improve the biocontrol efficacy of many commercially available mycopesticides based on these fungi and applied in a conventional manner, mainly as inundative sprays (Vega et al., 2009; Jaber and Ownley, 2017). Entomopathogenic fungi in several genera (e.g. *Beauveria*, *Metarhizium*, *Lecanicillium*, etc.) have been shown to negatively affect many herbivorous insects feeding on plants hosting them as endophytic microorganisms (Vidal and Jaber, 2015). Plant colonization with endophytic entomopathogenic fungi has been reported to reduce the damage caused by several insect pests such as *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) in maize, *Sesamia nonagrioides* Lefebre (Lepidoptera: Noctuidae) in sorghum, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) in banana, *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) in cotton, *Aphis fabae* Scopoli (Hemiptera: Aphididae) and *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae) in broad bean, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in onion, *Myzus persicae* Sulzer (Hemiptera: Aphididae) in pepper, in addition to many others (recently reviewed in Jaber and Ownley, 2017). Application of fungal entomopathogens as endophytic biocontrol agents might help circumvent many of the shortcomings associated with their application as inundative biocontrol agents (Vidal and Jaber, 2015). As endophytes, entomopathogenic fungi are less exposed to damaging UV radiation and other adverse microclimate conditions (Jaber and Ownley, 2017), more compatible with other groups of natural enemies (Akutse et al., 2014; Jaber and Araj, 2017), and more capable of targeting pests that would otherwise be difficult to control due to their cryptic or lower leaf surface feeding habits (Jaronski, 2010). Furthermore, the combined use of endophytic entomopathogenic fungi with other compatible products (e.g. insecticides) might produce higher control efficacy levels (Gurulingappa et al., 2011a).

Also used as alternatives to synthetic chemicals for pest management, botanical insecticides are plant-derived products with hundreds of identified and isolated active substances against a wide range of pests (Isman, 2006; Regnault-Roger and Philogène, 2008). Historically, plants have been long known to possess a chemical arsenal of secondary metabolites that limit damage inflicted on them by herbivorous insects and plant pathogens. Although academic interest and research activity in the field of botanical insecticides have grown dramatically over the past 30 years, such a surge in research on botanicals has not been translated into a corresponding progress toward commercialization of effective botanical-based pest control methods (Isman and Grieneisen, 2014). This could be partly due to tremendous competition with inexpensive and highly efficacious synthetic insecticides, especially those with newer chemistries such as the "reduced risk" pesticides (e.g. neonicotinoids); and also, to a lesser extent, with microbial-based biopesticides (Isman, 2006; Regnault-Roger and Philogène, 2008; Isman and Grieneisen, 2014). However, botanical insecticides can be better applied in combination or rotation with synthetic or microbial insecticides, rather than as stand-alone products (Isman, 2006). Indeed, compatibility of entomopathogenic fungal control agents with botanical products to manage insect pests such as whiteflies has been examined for neem (a steroid-like triterpenoid) extracted from the seeds of the Indian neem tree *Azadirachta indica* A. Juss (Meliaceae). Islam et al. (2010) found that an integrated application of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) with neem caused higher *B. tabaci* nymphal mortality level than individual treatments of *B. bassiana* and neem. Similarly, combining *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Deuteromycotina: Hyphomycetes) and neem (azadirachtin) yielded up to 90% *B. argentifolii* nymphal mortality, which was significantly higher than the mortality levels obtained when only one of the two agents was used (James, 2003).

Yet, compatibility of fungal entomopathogens with other botanicals, such as extracts of *Calotropis procera* (Aiton) W. T. Aiton (Asclepiadaceae) and *Inula viscosa* (L.) Aiton (Compositae), for whitefly management has not been demonstrated so far. Previous studies have reported the efficacy of *C. procera* in controlling several insect pests, including whitefly (Markouk et al., 2000; Ramos et al., 2006; Barati et al., 2013). When applied alone, *C. procera* extract showed insecticidal activity against *B. tabaci*; but was not as effective as the chemical insecticides tested in the same study for controlling this pest (Barati et al., 2013). On the other hand, the efficacy of *I. viscosa* extract against *B. tabaci* has never been examined before, but is already reported against other insect pests and plant diseases (Qasem et al., 1995; Mamoci et al., 2012; Seca et al., 2014). Also noteworthy is the fact that no studies to date have ever explored the combined effects of these or other plant extracts and entomopathogenic fungi for pest control when the fungi are endophytic. Moreover, even though the detrimental effects of endophytic fungal entomopathogens against pests have been reported for an increasing number of herbivorous insects (Vidal and Jaber, 2015; Jaber and Ownley, 2017), they have not been previously reported for the survival and development of different life stages of *B. tabaci* (but see Garrido-Jurado et al., 2017). Therefore, using potted cauliflower (*Brassica oleracea* L.) plants, we tested for the first time the effects of endophytic plant colonization by fungal entomopathogens (*B. bassiana* and *Metarhizium brunneum* Petch; Ascomycota: Hypocreales), alone and in combination with the aqueous extracts of two wild grown medicinal plants (*C. procera* and *I. viscosa*), on the survival and development of *B. tabaci*; and determined whether these effects are additive, synergistic, or antagonistic.