Potential of Two Entomopathogenic Fungi, Beauveria bassiana and Metarhizium anisopliae (Coleoptera: Scarabaeidae), as Biological Control Agents Against the June Beetle

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RESEARCH

Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle

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ABSTRACT. The aim of this study was to evaluate the effectiveness of the entomopathogenic fungi (EPF), *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) strain PPRI 5339 [BroadBand, an emulsifiable spore concentrate (EC) formulation] and *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae) strain F52 [Met52, both EC and granular (GR) formulations] against the larvae of *Polyphylla fullo* (L.) (Coleoptera: Scarabaeidae). Larvicidal bioassays were performed in foam boxes (100 by 75 by 50 cm; length by width by height), containing moist soil medium with some humus and potato tubers as food. Although the *B. bassiana* product (min. 4×10^9 conidia/ml) was applied at 100, 150, and 200 ml/100 l water; *M. anisopliae* strain F52 was applied at 500, 1,000, and 1,500 g/m^3 of moist soil medium for GR (9×10^8 cfu/g) and 75, 100, and 125 ml/100 l water for EC (5.5×10^9 conidia/ml) formulation. Both fungi were pathogenic to larvae of the pest; however, young larvae (1st and 2nd instars) were more susceptible to infection than older ones (3rd instar). Mortality rates of young and older larvae varied with conidial concentration of both fungi and elapsed time after application. The *B. bassiana* product was more effective than both of the formulations of the *M. anisopliae* product, causing mortalities up to 79.8 and 71.6% in young and older larvae, respectively. The highest mortality rates of young and older larvae caused by the *M. anisopliae* product were 74.1 and 67.6% for the GR formulation, 70.2 and 61.8% for the EC formulation, respectively. These results may suggest that both fungi have potential to be used for management of *P. fullo*.

Key Words: entomopathogenic, fungi, microbial

The family Scarabaeidae (Insecta: Coleoptera) consists of over 30,000 species of beetles worldwide. The species in this family are often called "scarabs" or "scarab beetles," and their larvae are generally known as "white grubs". *Polyphylla* Harris, 1841 is a genus of scarab beetles, including about 45 species distributed in North and Central America, southern and central Europe, northern Africa and southern Asia—from Asia Minor to Japan (Borror et al. 1981, Harde 1984, Chinery 1993, Csoka and Kovacs 1999). At least three species of this genus, namely *Polyphylla fullo* (L.), *P. olivieri* Cast., and *P. turkmenoglui* Petr., are recognized in Turkey; however, the first one is considered the most common and to be the only species of economic importance in many districts of Turkey (Turkmenoglu 1967). Depending on crop grown and soil type, the damage rate of this species in Turkey varies between 50 and 80%, but from time to time, in the vineyards grown in sandy soil 100% damage can occur (Anonymous 2011a). In the vineyards or young fruit orchards infested with this pest species, a pesticide application is advised when detected 1–2 larvae (young or older) per plant root (Anonymous 2011b). In lawn and turf areas, an approximate economic threshold for this species is 8–10 grubs per square meter (Anonymous 2008). Although being reported that the presence of the species in all parts of Turkey, there is no information on the hectares infested, hectares treated with insecticides, etc.

The June beetle, also known as "pine chafer," *P. fullo*, has a biennial or triennial life cycle by region in Turkey. Adult beetles emerge during June or July and fly evenings and nights. They feed on the leaves and needles of trees and then lay eggs in the soil. The larvae feed during the late summer and early fall and move deep in the soil to overwinter. After the hibernation, they return to the root zone and feed throughout the following summer. The larvae cause most injury during this second season of their life cycle. During spring and early summer of the next year, the larvae complete development, cease feeding, and turn into pupae and adults that remain inactive in the soil. Adult beetles emerge next season. In cold regions of the country, the larvae overwinter three times in the soil then pupate in May of the third year; the adults appear from mid-June to mid-July, feed then lay eggs (Anonymous 2008, 2011a,b).

P. fullo is one of the most damaging pests of young fruit orchards, vineyards, ornamentals, turfgrass, potatoes, and many other crops in southwestern Turkey (Antalya) (Bodenheimer 1958, Anonymous 2011a). The C-shaped larvae of the pest, also called white grubs, live in the soil (especially, in sandy habitats), often in the top 15–30 cm soil surface layer of the root zone during warm months. They generally are creamy white with three pairs of legs and attack the root system of many crops, including young fruit trees, grape vines, turfgrass, and ornamentals. Young larvae feed on humus and the roots of herbaceous plants; older larvae gnaw through the roots of shrubs and trees, and severe feeding injuries result in wilting and often death of infested plants (Davidson and Lyon 1979, Borror et al. 1981, Woodruff and Beck 1989, Buss 2006). In Antalya, considerable economic damage to vineyards and almond, cherry, prune, and apple orchards can occasionally be caused by the feeding of the June beetle larvae. Control of the beetle in heavily infested orchards requires that the infested trees be removed and the soil fumigated. This procedure is extremely expensive and destructive, and removes the orchard from production for a number of years. The species has recently become a serious pest of turfgrass in many touristic places, parks and gardens in Antalya (F. E. et al., unpublished data).

Control measures include the use of high-quality seeds and planting stock, treatment of seedling roots or furrows and plantings with insecticides, direct application of insecticides to the soil, and leaving the soil fallow and treating it repeatedly with insecticides when the pests are most numerous (Tashiro 1973, Metcalf and Metcalf 1993, Buss 2006). There are some insecticides registered worldwide for use against white grubs. White grubs, however, are among the most difficult soil insect

pests to control and highly resistant to insecticides (Buss 2006). More importantly, because grubs feed in the soil, it is difficult to get adequate amounts of insecticide into the root zone. Under typical conditions, control is often <75%. Even this amount of control requires a couple of weeks to become evident. In Turkey, previously used chemical insecticides in white grubs' control have recently been phased out and are not currently available for purchase. Development of resistance, withdrawal of registered chemicals from the market and limited prospects for the registration of new materials has severely reduced white grub control options. Depending on its origin (biological or chemical), a period of 3–6 yr is required for a new pesticide registration in Turkey. Although the regulatory system of pesticides in Turkey encourages the development and use of innovative and lower-risk products, to get registration for a new biological product (microbial or botanical origin) requires at least 3 yr. Like in many districts of Turkey, in the Antalya district, white grub control is commonly based on repetitive applications of chemicals (none of them are currently registered), resulting in environmental pollution and resistance in pest populations. In addition, excessive use of chemical insecticides can result in high residues in the soil that are dangerous for non-target organisms and the environment. Owing to high residues and toxicity of common insecticides to non-target organisms, their application to many crops has recently been limited or strictly restricted in Turkey. Thus, white grub control encounters a great challenge and interest in utilizing new control agents.

An attractive alternative to chemical pesticides is microbial control agents (MCAs), especially entomopathogenic fungi (EPF), with no or low-hazard effects on human health and environment. Most EPF belong to the orders of the Hyphomycetes or Entomophthorales (Butt et al. 2001). Some fungi from Hyphomycetes were the first described to cause death in some insect species. For example, Agostino Bassi first suggested that a microorganism caused the "white muscardine" disease in silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) in 1835. The causative fungus species was later named *Beauveria bassiana* (Balsamo) Vuillemin in his honor (Steinhaus 1963). Metchnikoff produced large quantiies of the "green muscardine", *Metharizium anisopliae* (Metsch.) Sorokin spores and attempted to control the wheat cockchafer, *Anisoplia austriaca* Herbst (Coleoptera: Scarabaeidae) using this fungus in Russia in 1879 (McCoy et al. 1988). *M. anisopliae* has been registered as a commercial product against grasshoppers, >100 yr after Metchnikoff's first trials (Milner and Hunter 2001). A different type of "white muscardine" was observed to attack the European cockchafer, *Melolontha melolontha* (L.) (Col.: Scarabaeidae), forming dense white layers on the insect's cadaver. Dufour (1894) for the first time described a host's population collapse, i.e., an epizootic in *Me. melolontha*, caused by an EPF. The causative agent, the "white muscardine," was identified as *Beauveria tenella* (Saccardo) D.M. Macleod., later named *Beauveria brongniartii* (Saccardo) Petch (De Hoog 1972). Keller and Zimmermann (1989) reported that the order, Hyphomycetes typically induce epizootics in populations of soil dwelling insects. The MCAs are increasingly seen in the European Union (EU) as important crop protection tools, and those registered in Europe are listed (Annex I) (Keller 2000, Keller and Zimmermann 2005, Kabaluk et al. 2010). Currently, available fungal products belong to the Hyphomycetes genera *Beauveria*, *Metarhizium*, *Verticillium*, *Paecilomyces*, and *Trichoderma* and may have different names in different states in the EU (EPA 2007, European Union 2007). Of these, the two fungal species *B. brongniartii* and *M. anisopliae* are applied as MCAs below ground: the *B. brongniartii* based products BeauveriaSchweizerTM (Switzerland), EngerlingsgspilzTM (Switzerland), MELOCONTTM-Pilzgerste (Austria and Italy), and BetelTM (France) are applied against the European cockchafer, *Me. melolontha*. All these fungal products are based on cereal grains overgrown with fungal conidia spores (Keller et al. 2002). In other parts of the world, for example in Japan, BiolisaKamikiriTM, containing *B. brongniartii* is registered to control scarabidaen garden- and forest-pest beetles (Higuchi et al. 1997). In Australia, the commercial product BioGreenTM, based on *M. anisopliae*, is registered for use against the red-headed pasture cockchafer (Rath et al. 1995). The present study was aimed at investigating the effectiveness of an emulsifiable concentrate (EC) formulation of *B. bassiana* strain PPRI 5339 (BroadBand) and two different formulations, granular (GR) and EC, of *M. anisoplia* strain F52 (Met52) on the mortality of *P. fullo* larvae.

Materials and Methods

Test Materials

Samples of the *B. bassiana* strain PPRI 5339 product (BroadBand EC), containing min. 4×10^9 conidia/ml, were provided by Bioglobal Inc. (Konyaalti St. 07100 Antalya, Turkey; http://www.bioglobal.com.tr/tr) via Becker Underwood (West Sussex, UK; http://www.beckerunderwood.com/). Samples of both GR and EC formulations of the *M. anisopliae* strain F52 product (Met52) were provided by Thierry Pradier (Novozymes Biologicals, 60 Route de Sartrouville, 78230 Le Pecq., France; http://www.novozymes.com/). The EC formulation of Met52 contains 5.5×10^9 conidia/ml, and *M. anisopliae* strain F52 spores are suspended in an emulsifiable oil as a formulation suitable for spraying or drenching into soil-like a chemical insecticide. The GR formulation of Met52 is composed of spores of *M. anisopliae* strain F52 on a grain matrix, containing 9×10^8 cfu/g.

To further identify the effectiveness of these three fungal formulations on *P. fullo* larvae, their effects were compared with that of a standard pesticide, chlorpyrifos-ethyl (Dursban 4 EC 480 g/l; Dow AgroSciences, Antalya, Turkey; http://www.dowagro.com/turkey/), one of the most commonly used pesticides for control of white grubs in Turkey. However, the use of some organophosphates, including chlopyrifos (-ethyl and -methyl forms), has newly been phased out in Turkey.

Insect Material

All insects used in this study were obtained from a stock culture of *P. fullo* maintained in the laboratory of Bioglobal Inc. The June beetle larvae at different stages were collected from heavily infested areas in the Antalya-Elmalı district during soil ploughing in the summer of 2011. They were transferred into the foam boxes (100 by 75 by 50 cm; L by W by H), containing moist soil medium with some humus and potato tubers as food, and then transported to the laboratory in Bioglobal Inc.

In the laboratory, collected larvae were morphologically identified to species level under a stereo-microscope (4×–25×) with characteristics at the end of the abdomen (Schwenke 1974), and only those of *P. fullo* were used in bioassays. As the larvae of *P. fullo* are obligate phytophag/xylophag (Tashiro 1973, Csoka and Kovacs 1999), they were supplied with potato tubers by inserting into the soil medium in the foam boxes every week. Now and then, the soil surface was sprayed with tap water to maintain humidity of soil medium in the boxes at a level similar to that of realistic field conditions. After a 2-wk supply of food, all the larvae were used in bioassays. A voucher specimen of collected larvae was deposited at the Plant Protection Department of Akdeniz University (Antalya, Turkey), under the catalogue number, 2011/Scarab-22.

Test Procedure and Larvicidal Bioassays

Experiments were conducted in the laboratory of Bioglobal Inc. Foam boxes (100 by 75 by 50 cm; L by W by H), each including ~0.02 m^3 (~25 kg) of moist soil medium, were used as test medium. Because white grubs seem to prefer soils with sandy or loamy sand textures (Anonymous 2011a), the soil used in the study was sandy-loam being representative of the sandy-loam texture soils of the Antalya region with pH: 7.9, lime: 6.17%, electrical conductivity (EC; mmhos): 2.5, organic matter: 1.88%. Each box was considered as one replicate, and two replicates were used for each treatment (for each concentration × exposure time combination). Each replicate set contained two control boxes including larvae that had been treated with tap water only

(negative control) and two other boxes treated with chlorpyrifos-ethyl (positive control) too. As the experiments were repeated twice, thus the total number of replicates for each concentration × exposure time combination was four.

Bioassays were performed using methods of Fleming (1960) and Tashiro (1973) with minor modifications. First, *P. fullo* larvae obtained from the stock culture were separated into two groups, young (1st and 2nd instars) and older (3rd instar) ones, according to head capsule width. Fifteen young or older larvae were introduced into each box, containing moist soil medium with some humus and potato tubers as food, which was considered as one replicate, and then exposed to any concentration of fungal products or the standard pesticide, chlorpyrifos-ethyl, used for comparison. Although the *B. bassiana* EC product was applied at 100, 150, and 200 ml/100 l water; *M. anisoplia* strain F52 was applied at 500, 1,000, and 1,500 g/m^3 of moist soil medium for the GR formulation (that is recommended at a label rate of 60–122 kg/ha when applying to field-grown crops) and 75, 100, and 125 ml/100 l water for the EC formulation. According to another calculation, the doses applied were 2.4×10^4, 3.6×10^4, and 4.8×10^4 conidia per gram soil for *B. bassiana* EC product; 3.6×10^4, 7.2×10^4, and 10.8×10^4 for the GR formulation of *M. anisopliae* strain F52; and 2.2×0^4, 2.9×10^4, and 3.7×10^4 for the EC formulation of *M. anisopliae* strain F52 product. All these doses were within the manufacturers' recommended ranges. Chlorpyrifos-ethyl (positive control) was applied at the recommended label rate (150 ml/100 l water) in all experiments. All EC products were applied as dilute sprays (1,500 ml per box) using a handheld sprayer with a tank capacity of 5 liters. All of the EC treatments were applied by soil drench for sufficient wetting of the top 10–15 cm soil surface layer where larvae are found. However, separate sprayers were used for each product to prevent cross-contamination. The GR formulation of *M. anisopliae* was applied by thoroughly mixing the product into the soil medium in the boxes, ensuring even distribution. Soil was moist at the time of application and maintained in a moist condition after application for best performance. Tap water alone was applied to the negative control boxes.

Environmental factors like temperature, humidity, and sunlight play a profound role on field persistence of EPF (especially, at the beginning of their growth, sporulation, and infection to the cuticle of host insect pests), and this period lasts ~3 to 8 h for many EPF (Vidal and Fargues 2007). So, the boxes were kept away from direct sunlight ~8 h after application and kept under the controlled conditions [26 ± 2 °C and 75–80% relative humidity (RH)] in a particular part of the laboratory throughout the study. These temperature and RH values are similar to those of Antalya in late spring/early summer.

Data Collection and Statistical Analysis

The efficacy of the treatments was evaluated by counting young and older larvae of the pest, live or dead, after each exposure time. The number of living and dead larvae per box was recorded each counting time. The counts were made on the 3rd, 7th, 14th, and 21st d after application. At each counting time, the boxes were poured onto a nylon sheet and the larvae were collected from the soil medium using a soft insect forceps. The larvae were considered dead if they did not move when prodded with a dissecting needle. During the data collection, especially 7, 14, and 21 d after application, infected larvae conspicuously shrunk, turned orange-brown for *B. bassiana* or dark-gray for *M. anisopliae* and had more or less fungal outgrowths (with a white fuzz appearance) on the surface. Microscopic examination showed that *B. bassiana* colonies are usually slow growing, downy, at first white but later often becoming orange-brown and its conidiogenous cell with globose bases and extended, denticulate rachis and the conidia are globose in shape (< 3.5-µm diameters). As to *M. anisoplia*, colonies are pale to bright green to yellow-green but later dark-gray and mycelium often wholly covering affected hosts; conidiophores in compact patches; individual conidiophores broadly branched (candelabrum-like), densely intertwined; conidiogenous cells with rounded to conical apices, arranged in dense hymenium; conidia aseptate, cylindrical or ovoid, forming chains usually aggregated into prismatic or cylindrical columns or a solid mass of parallel chains. Conidia are 7–9 µm long.

The efficiency of the products tested was calculated according to the following formula described by Henderson and Tilton (1955), with minor modifications.

$$Efficiency\,(\%) = [1 - (A1 \times B1/A2 \times B2) \times 100]$$

where $A1$ = number of living larvae in the treated box after treatment,
$A2$ = number of living larvae in the treated box before treatment,
$B1$ = number of living larvae in the water-treated control box after treatment,
$B2$ = number of living larvae in the water-treated control box before treatment.

Mortality data obtained from the study were normalized by arcsine square-root transformation and subjected to analysis of variance (ANOVA). Untransformed means are presented here. Significant differences among the treatment means were separated using the Duncan's multiple range test (DMRT), and a probability (P) of ≤ 0.05 was accepted as statistically significant (SPSS 17.0).

Results

The results from the study showed that all of the fungal products tested, EC formulation of *B. bassiana* strain PPRI 5339, and both GR and EC formulations of *M. anisoplia* strain F52, had different efficacy rates against different larval stages of *P. fullo*. For each fungal product, mortality rates of young and older larvae of the pest were significantly different at different conidial concentrations (DMRT, $P \leq 0.05$), and efficacy generally increased with increasing concentration and elapsed time (up to 2 wk after application). The mortality rates of young and older larvae of *P. fullo* are listed in Tables 1 and 2.

All the fungal products at the three conidial concentrations and the standard pesticide, chlorpyrifos-ethyl, used for comparison yielded significantly higher mortality rates of both young and older larvae of the pest than the water-treated control (DMRT, $P \leq 0.05$). However, young larvae were more susceptible to all the products tested than older ones. Mortality rates of different larval stages caused by fungal infection generally varied over time (up to 14 d after application), and differences of the mortalities at different exposure times were generally higher among the different conidial concentrations of all the fungal products and the standard pesticide tested (DMRT, $P \leq 0.05$). The mortality rates of young and older larvae caused by all of the fungal treatments 3 and 7 d after application were lower than those by the standard pesticide, however, 14 and 21 d later significantly higher mortality rates were achieved by the fungal products compared with the standard pesticide (DMRT, $P \leq 0.05$). Whereas the standard pesticide achieved the highest mortality rate of young and older larvae (67.8 and 58.6%, respectively) on the 7th d after treatment, the highest rates caused by the fungal products occurred 14 or 21 d after treatment. The mortality rates of both young and older larvae caused by all of the concentrations of each fungal product did not differ significantly from each other on the 14th and 21st d after treatment (DMRT, $P \leq 0.05$). The same condition was also valid for the young and older larval mortalities caused by the standard pesticide on the 7th, 14th and 21st d after treatment.

When all three fungal formulations tested were directly compared with one another in terms of the mortality rates of both young and older larvae of the pest, the EC formulation of *B. bassiana* strain PPRI 5339 was the most effective against both young and older larvae, causing mortality 79.8 and 71.6%, respectively. The highest mortality rates of young and older larvae caused by *M. anisopliae* strain F52 were 74.1 and 67.6% for the GR formulation and 70.2 and 61.8% for the EC formulation, respectively. The mortality rates of both young and older

Table 1. Mortality rates of young (1st and 2nd instars) larvae of *Polyphylla fullo* in response to the products tested 3, 7, 14, and 21 d after application (A) under laboratory conditions*

Test materials and concentrations used (ml/100 l water or g/m^3 of soil medium)	Mean percent mortality (±S.E.) after application A+3	A+7	A+14	A+21
Beauveria bassiana (EC)				
100	12.5 ± 3.2B^{y}a^z	31.8 ± 4.4 BCb	48.2 ± 4.6 Cc	51.6 ± 4.9 Cc
150	23.8 ± 4.1 Ca	42.4 ± 5.8 DEb	66.9 ± 5.3 EFc	69.8 ± 5.2 EFc
200	27.2 ± 6.2 Ca	46.3 ± 5.2 Eb	79.8 ± 5.8 Gc	76.2 ± 5.9 Fc
Metharizium anisopliae (EC)				
75	9.6 ± 3.6 Ba	32.6 ± 4.9 BCb	36.7 ± 4.3 Bb	37.8 ± 4.6 Bb
100	14.8 ± 4.3 Ba	29.7 ± 3.3 BCb	51.4 ± 5.5 CDc	54.4 ± 5.2 CDc
125	16.2 ± 4.7 Ba	37.8 ± 4.1 CDEb	70.2 ± 6.4 Fc	68.6 ± 6.0 EFc
M. anisopliae (GR)				
500	11.3 ± 3.6 Ba	26.3 ± 4.8 Bb	46.6 ± 4.5 Cc	47.3 ± 4.1 BCc
1,000	14.8 ± 4.3 Ba	28.7 ± 3.2 BCb	59.8 ± 5.1 DEc	63.4 ± 5.6 DEc
1,500	22.7 ± 4.7 Ca	35.2 ± 4.1 BCDb	71.3 ± 5.8 FGc	74.1 ± 6.1 EFc
Positive control (chlorpyrifos-ethyl)^x	46.4 ± 5.9 Da	67.8 ± 6.2 Fb	64.3 ± 7.1 EFb	65.0 ± 6.2 Eb
Negative control (tap water)	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	2.6 ± 0.8 Aa	3.2 ± 0.8 Aa

*Values are means of four replicates. xThe standard insecticide, chlorpyrifos-ethyl, used for comparison was applied at the recommended label rate (150 ml/100 l water). yMeans within a column followed by the same capital letter are not significantly different (DMRT, $P \leq 0.05$). zMeans within a row followed by the same lower-case letter are not significantly different (DMRT, $P \leq 0.05$).

Table 2. Mortality rates of older (3rd instar) larvae of *P. fullo* in response to the products tested 3, 7, 14, and 21 d after application (A) under laboratory conditions*

Test materials and concentrations used (ml/100 l water or g/m^3 of soil medium)	Mean percent mortality (±S.E.) after application A+3	A+7	A+14	A+21
B. bassiana (EC)				
100	8.6 ± 3.3BCD^{y}a^z	19.3 ± 3.7 Bb	27.4 ± 3.6 Bb	28.1 ± 4.3 BCb
150	14.1 ± 3.9 CDa	32.4 ± 3.8 Cb	49.5 ± 5.2 DEc	51.2 ± 6.2 DEc
200	17.5 ± 5.6 Da	41.6 ± 5.6 Db	71.6 ± 6.5 Gc	70.1 ± 5.6 Gc
M. anisopliae (EC)				
75	3.6 ± 1.8 ABa	13.2 ± 3.4 Bab	23.7 ± 4.5 Bb	21.3 ± 3.9 Bb
100	7.8 ± 2.6 ABCa	19.3 ± 4.2 Bb	35.1 ± 4.8 BCc	39.5 ± 5.1 CDc
125	11.2 ± 3.3 BCDa	31.7 ± 4.1 Cb	59.4 ± 6.4 EFc	61.8 ± 5.6 EFGc
M. anisopliae (GR)				
500	8.9 ± 2.3 BCDa	17.2 ± 3.7 Bb	24.8 ± 4.1 Bbc	26.0 ± 3.4 Bc
1,000	12.4 ± 3.4 CDa	28.6 ± 5.2 Cb	44.1 ± 5.1 CDc	43.6 ± 4.5 Dc
1,500	11.7 ± 4.1 BCDa	30.5 ± 4.4 Cb	65.9 ± 4.8 FGc	67.6 ± 5.7 FGc
Positive control (chlorpyrifos-ethyl)^x	38.4 ± 6.4 Ea	58.6 ± 6.0 Eb	56.3 ± 6.2 EFb	57.1 ± 6.1 EFb
Negative control (tap water)	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	3.7 ± 0.7 Aa	2.6 ± 0.8 Aa

*Values are means of four replicates. xThe standard insecticide, chlorpyrifos-ethyl, used for comparison was applied at the recommended label rate (150 ml/100 l water). yMeans within a column followed by the same capital letter are not significantly different (DMRT, $P \leq 0.05$). zMeans within a row followed by the same lower-case letter are not significantly different (DMRT, $P \leq 0.05$).

larvae, in general, differed significantly among the different conidial concentrations (except for the lower concentrations) of all the fungal formulations tested (DMRT, $P \leq 0.05$). Thus, all the fungal products varied in ability to infect *P. fullo* larvae, and their impact on mortality largely depended on the conidial concentrations applied and the elapsed time after application.

Discussion

Recently, white grub management in Turkey has become increasingly difficult because of development of insecticide resistance, withdrawal of registered chemicals from the market, increasingly stringent government regulations of pesticides. Damage and losses on crops at localities with insecticide-resistant white grub populations have steeply increased recently and pest control efforts have become inefficient. Many control programs are now aimed at holding white grub populations at low levels throughout the growing season. Populations that are allowed to reach high levels become difficult to control. Traditionally, initial control efforts in many parts of Turkey have involved several applications of synthetic insecticides. However, resistance to insecticides has reduced the effectiveness of this approach, and alternative control methods and materials are needed. Although there have been reported some natural enemies of white grubs in Turkey (Turkmenoglu 1967, Karagoz et al. 2011), none of them has been practiced in management. As a matter of fact, due to excessive use of pesticides against white grubs, we cannot rely on natural enemies without using alternative control strategies to prevent damage by white grubs.

The results of the current study showed that the EPF, *B. bassiana* strain PPRI 5339 and *M. anisopliae* strain F52, were effective against *P. fulo* larvae, and comparable in efficacy to the standard pesticide, chlorpyrifos-ethyl. In the laboratory bioassays, respectable rates of mortality on *P. fulo* larvae (>70% mortalities, especially in young, 1st and 2nd instars, larvae of the pest) were obtained from *B. bassiana* and *M. anisopliae* applied within label rates. A review of literature concerning EPF revealed that there are some works in Europe and other parts of the world on their effects against various scarab beetles, e.g., *Melolontha* spp., *Phyllopertha* spp., *Hoplia* spp., and other soil pests like *Otiorhynchus* spp., but no field or laboratory trials of the two EPF, *B. bassiana* and *M. anisopliae*, have so far been performed with the June beetle or Pine chafer, *P. fullo*, larvae. Benker and Leuprecht (2005) reported that Common cockchafer *Me. melolontha* is the main scarab species in the Bavarian region, Spessart (in Germany), followed by the Summer chafer *Amphimallon solstitiale* L., the Welsh chafer *Hoplia philanthus* (Fuessly) and the Garden chafer *Phyllopertha horticola* (L.), and tried an EPF (*B. brongniartii*) and two insecticides (Imidacloprid and Carbofuran) in the control of *Me. melolontha*. Both Carbofuran and Imidacloprid provided quite good results, even in the

plots treated with Imidacloprid nearly no grubs could be found at the end of study, i.e., 8 wk after application. The efficacy of the *Beauveria* fungus was slow and not satisfactory, but in the end there was a decrease of 80% of the starting number of the grubs. Despite of the good results of both insecticides, they recommend using *Beauveria* fungus for the long-term control strategy of grubs. Pernfuss et al. (2005) reported relatively less effective control of a scarab species, *P. horticola*, treated with a new product based on *M. anisopliae* (Granmet-P). Eight weeks post treatment the pest was reduced between 14 and 16% in the plots where the *M. anisopliae* product (Granmet-P) was used, whereas the chemical insecticide, chlorpyrifos (Dursban-2E), used for comparison, caused 35% larval mortality within the same span of time. In screening and selection tests with virulent isolates of the EPF *B. brongniartii* under laboratory conditions, 10 *B. brongniartii* isolates, obtained from different geographical regions and hosts in Europe, were tested against the larvae of scarabs, *Me. melolontha* and *Holotrichia serrata* L., and at a concentration of 2×10^7 conidia/ml after 30 d, all isolates of *B. brongniartii* were found to be pathogenic to third instar larvae of *Me. melolontha* and *H. serrata* with differences in their virulence; three isolates Bbr 50, Bbr 23, and ARSEF 4384, causing 95.83, 83.34, and 79.17% mortality, respectively, for *Me. melolontha* and two isolates ARSEF 4384 and ARSEF 2660, causing 75.56 and 54.00% mortality, respectively, for *H. serrata* were shown to be more pathogenic in terms of total mortality, onset of mortality and mycosis (Hadapad et al. 2005). Berón and Díaz (2005) reported that different isolates of *B. bassiana* were generally more virulent to most soil-dwelling insect pests than *M. anisopliae*. This is consistent with the results reported here. However, higher virulence of *M. anisopliae* compared with *B. bassiana* isolates against *Anomala cincta* (Say) (Coleoptera: Melolonthidae) was found by Guzmán-Franco et al. (2012). Also, Klein et al. (2000) suggest that *Metarhizium* species are better adapted to infect soil-dwelling insects than *Beauveria* species as they have been more commonly found causing infection on soil pests. It is therefore difficult to say anything definite about comparative effectiveness of both EPF against soil-dwelling insect pests.

Different biotic and abiotic conditions (host species, abundance of host, application timing, rate, delivery of product to the target area, moist, temperate climate, rainfall, soil-covering index, edaphic factors, etc. may all be factors that could be causing the inconsistent results with *B. bassiana* and *M. anisopliae* for grub control (Keller et al. 1997, Inyang et al. 2000, Kessler et al. 2003, Strasser et al. 2005, Bugeme et al. 2008, Sharififard et al. 2012). Additionally, Vidal and Fargues (2007) reported that growth, sporulation, infectivity and survival of EPF are greatly affected by temperature, RH and solar radiation. Southwestern Turkey, where the study was performed, has a Mediterranean climate, characterized by warm to hot, dry summers and mild to cool, wet winters. Temperature increases gradually during the spring months while humidity decreases. When soil temperature gets too hot; i.e., exceeds the optimum growing temperatures that are in the range of 18–30 °C for both EPF tested, it is obvious that the effectiveness of these two fungi will decrease. That is why it is necessary that such applications be made at a time when soil temperature and humidity is suitable for optimal entomopathogenic activity.

The results of the current study also indicate that young larvae are more susceptible to the fungal products tested than older ones. That is why it is necessary that applications be made at a time when the majority of larvae are at a susceptible stage of development. Also, Van Steenwyk et al. (1990) report that controlling the adult 10 lined June beetles in almonds with foliar insecticides seems unlikely because of the prolonged adult male emergence and female behavior after mating, and suggest the most suitable stage for control is young larvae while they are actively feeding in the soil.

Integration of EPF in the integrated pest management (IPM) strategy for control of white grubs can reduce reliance on synthetic insecticides and increase the levels of control especially against early season white grub populations. EPF are also ready-made components of IPM because of their complementary or synergistic insecticidal activity with other control elements including predators and parasitoids (Roy and Pell 2000; Lacey et al. 2001; Wraight et al. 2001; Goettel et al. 2000, 2010). Commercial products based on *B. bassiana* and *M. anisopliae* are currently in use in some parts of the world like Europe, United States, Australia etc. or under development. Faria and Wraight (2007) gave a comprehensive list with worldwide coverage and international classification of formulation types of the EPF *B. bassiana* and *M. anisopliae* strains were used successfully in controlling different insect pests under field conditions (Puterka 1999; Lababidi 2002; Lacey et al. 2001, 2011).

Based on the results of this study, it was concluded that the EPF products tested may provide viable alternatives to synthetic insecticides used in the control of white grubs. Their use together with other MCAs like parasitic nematodes (*Heterorhabditis* spp., etc), as well as in conjunction with good agricultural practices (good hygiene, preservation of biological control agents, good cultural practices including the use of high-quality seeds and planting stock, weed sanitation, adequate nutrition and irrigation, soil ploughing in hot and dry seasons etc.), may reduce the use of chemical pesticides and provide an element within an IPM system.

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