Evaluation of Microbial Products for the Control of the Mushroom Phorid Fly, *Megaselia halterata* (Wood)¹

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Mushroom production in Turkey is normally divided into two parts—compost production and mushroom cultivation. Total fresh mushroom production of Turkey has increased more than 28-fold in the last 22 yrs from about 1400 tons in 1983 to about 40,000 tons in 2005. The district of Antalya-Korkuteli (southwestern Turkey) alone produces more than 50% of the total compost produced and about 45% of the fresh mushrooms sold in the country (Ozcatalbas et al. 2004, Anonymous 2007). The white button mushroom, *Agaricus bisporus* (Lange) Imbach (Agaricaceae), is the most commonly grown mushroom in the country, accounting for up to 95% of the total mushroom production (Erkel 2004, Anonymous 2007).

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Abstract Over the last decade, mushroom production has become one of the most actively developing fields of agriculture in Turkey. About 45% of the total mushroom production and >50% of the total compost production occurs in the Antalya-Korkuteli district (southwestern Turkey). Major insect pests of mushroom production are cecidomyiid, sciarid and phorid flies with Megaselia halterata (Wood) (Diptera: Phoridae) being the most common species in the district. In the present study, two commercial microbial products [a bacterial larvicide, Bacillus thuringiensis var. israelensis Berliner (Bti) commercially available as Gnatrol® (Valent USA Corp., Walnut Creek, CA), and an entomopathogenic nematode, Steinernema feltiae (Filipjev) Wouts, Mracek, Gerdin & Bedding commercially available as Entonem® (Koppert Biological Systems, The Netherlands)] and spinosad, a biologically-derived insecticide that is commercially available as Laser® (Dow AgroSciences, Zionsville Road, IN), were evaluated for control of *M. halterata* in 3 successive mushroom-growing periods. These products were compared with a control treated with water and a conventional chemical insecticide control (chlorpyrifos-ethyl). Treatments were targeted at larvae as soil drenches; treatment efficacy was evaluated by assessing adult emergence and larval damage. Treatments with the microbial products had significantly lower numbers of emerging adults than those observed in water-treated control. There were no significant differences in adult emergence among the 3 microbial products and the chlorpyrifos-ethyl control over the 3 growing periods. Each of the microbial products reduced the incidence of fruit damage by the larvae and resulted in significantly lower damage rates when compared with the watertreated control. These results suggest that these microbial products can be used as alternatives to conventional chemicals in controlling *M. halterata* on mushroom.

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Mushroom flies (Diptera), including cecidomyild, phorid and sciarid species, are the primary insect pests of mushrooms. These pests are distributed in many parts of the world, including Asia, Australia, Europe and North America (Clift 1979, Wetzel 1981, White 1985, Kim and Hwang 1996). In Turkey, mushroom flies have recently received attention as pests. Their taxonomy, geographic distribution, life cycle, and some physiological aspects have been studied. Crop losses caused by these flies are estimated as at least 20% (Civelek and Onder 1996, Erkel 2000, Erler and Vurus 2004). Mushroom flies inhabit mushrooms as well as rotting wood, decaying potato, and decomposing vegetables (Stamets and Chilton 1984, Lee et al. 1999). In mushroom production, infestation by adult flies generally occurs as the compost cools and during the introduction of spawn into the compost and casing (Jess et al. 2007). Females may lay eggs in the compost and casing layer and cause severe damage to mushroom. Damage occurs as larval feeding on the mycelium and tunneling into the caps and stems of the mushrooms, as well as adults transmitting disease agents, nematodes, mites, and other contaminants (Clift 1979, Clancy 1981, Wetzel 1981, Kim and Hwang 1996).

Control of mushroom flies is an important component of producing high yielding, quality crops of mushrooms. Unfortunately, the reliable control of these pests has been complicated by the development of resistance to pesticides in pest populations, toxicity of pesticides to mushroom mycelium, persistent pesticide residues in the compost/casing material, and concerns with worker exposure to toxic pesticides. Consequently, novel or alternative pesticides and/or methods of application are necessary for the continued production of commercial mushrooms.

In Turkey, conventional control of mushroom flies relies on good management (e.g., compost pasteurization, fly screening, room fumigation, and general hygiene) coupled with the use of conventional insecticides. The latter may be incorporated into either the compost or casing layer, watered on during cultivation, or applied as a wall or space spray to control adults. These measures can give adequate control when correctly practiced. However, insecticide resistance problems have been detected, and the use of certain pesticides has resulted in yield reductions (unpubl. data). Also, many insecticides used by mushroom growers exhibit broad-spectrum and long residual activity. To develop sustainable methods of controlling mushroom flies, new control agents need to be evaluated. Therefore, the goal of the present study was to evaluate the effectiveness of some selective commercial formulations against the mushroom phorid fly, *M. halterata*.

Materials and Methods

Test materials. Two commercially available microbial agents—the bacterium *Bacillus thuringiensis* var. *israelensis* Berliner (*Bt*) and the entomopathogenic nematode *Steinernema feltiae* (Filipjev) Wouts, Mracek, Gerdin & Bedding—and spinosad, a biologically-derived insecticide, were tested for efficacy in managing *M. halterata*. The *Bti* was formulated as Gnatrol[®] (Valent USA Corp., Walnut Creek, CA), *S. feltiae* was formulated as Entonem[®] (Koppert Biological Systems, The Netherlands), and the spinosad was formulated as Laser[®] (Dow AgroSciences, Zionsville Road, IN). The choice of these products for inclusion in the study was based on preliminary laboratory testing and their improved safety for consumers. Efficacy of microbial products was compared with that of a standard pesticide, chlorpyrifos-ethyl (Alban[®], Agrobest LTD, Aydin, Turkey), one of the most commonly used insecticides for control of the

mushroom flies in the Antalya-Korkuteli district. A nonchemical control with water only also was included.

The mushroom used in the study was a variety (A-15 smooth white) of the standard species, *A. bisporus*, which is a commonly grown variety for commercial production in the study area and is usually susceptible to phorid fly damage. Pasteurized and spawned compost placed in 40-cm polyethylene bags were used in the trials. The compost and casing material were supplied from commercial sources. Growing procedures were the same as those used commercially (Gunay 1995, Erkel 2000).

Insects. Insects used in the study were from colonies maintained by methods modified from Richardson and Hesling (1978). Adults of *M. halterata* were collected with aspirators from commercial mushroom-growing cellars in the Antalya-Korkuteli district. These were then transferred into 5-L plastic jars and transported to the insectarium in the Plant Protection Department of Akdeniz University, Antalya. As the larvae of mushroom phorid flies are obligate mycetobionts and directly reduce the growth of *A. bisporus* mycelium (Hussey 1959, White 1985), collected adults of *M. halterata* (20 pairs per tray) were introduced over the spawn-runned compost trays (50 × 50 cm) covered with fine gauze cage (30 cm high) and allowed to mate and oviposit for 96 h at $26 \pm 1^{\circ}$ C. The adults were subsequently removed, and all trays containing spawn with eggs were covered with Parafilm[®] (Pechiney Packaging, Menasha, WI) to maintain humidity in the trays at a level similar to that of mushroom-growing cellars. Adult flies collected 48 h after emergence were used for the experiments.

Experimental site and design. The study was conducted in a mushroom cellar on the Campus of Akdeniz University in 3 successive growing periods in 2006 and 2007. The growing cellar used was similar in design to those used commercially but smaller in capacity (dimensions: 4.5×3.0 m). A total of 30 polyethylene bags, each including approx. 10 kg of standard pasteurized and spawned compost, were used during each growing period. Each bag was considered as a replicate with 6 replicates for each treatment. As the experiments were repeated in 3 separate periods, the total number of replicates for each treatment throughout the study was 18.

Biological assays. Ten pairs (109:10°) of 48-h-old adults were introduced over the casing layer in each polyethylene bag covered with a transparent nylon gauze cage (30 cm high) and arranged in dome shape (Fig. 1A). The introduced adults were allowed to mate and oviposit for 96 h. All adults were subsequently removed, and treatments were applied as larvae began to eclose, as determined by microscopic examination of small amounts of growing substrate taken from at least 10 bags. Only one application was made in each growing period. Treatments were applied by soil drench to sufficiently wet the top 10 cm of soil surface layer where larvae are found. All test preparations were applied at their recommended label doses. Dilute sprays (150 ml per bag, corresponding to 1200 ml/m²) of the test materials were applied using a handheld compressed air sprayer with a tank capacity of 5 L. However, separate sprayers were used for each treatment to prevent cross contamination. Only tap water was applied to the water controls. During the experiments, all growing practices were applied like those in a commercial mushroom growing cellar in the Antalya-Korkuteli district.

Treatments were evaluated by counting the emerging adults per bag in each treatment and measuring larval damage (mean number of fruits damaged by the larvae per treatment) (Fig. 1B). At weekly intervals for 5 wks, emerging flies were collected with an aspirator equipped with a vacuum source and counted. Mushroom fruits were harvested 6 times (2 per flush) in each growing period, and the number of fruits damaged by larvae was recorded at each harvest.



Fig. 1. Polyethylene compost bags, covered with a transparent nylon gauze cage in dome shape, used in the study (A) and fruit damaged (in circle) by larval stages of *Megaselia halterata* (B).

Data analysis. Numbers of emerging adults (adult emergence was expressed as number per m²) and numbers of damaged fruit at harvest were analyzed by ANOVA (SAS Institute 2001). Where significant differences occurred among treatments, means were separated using the Student-Newman-Keuls (SNK) test with $\alpha = 0.05$. Larval damage was expressed as the percentage of damaged fruits for each treatment in each growing period, and the percentage damage incidence was determined using the formula:

% damage = (number of fruits damaged / total number of fruits) \times 100.

Mean percentages were subjected to square-root transformation before analysis. Treatment means were separated by the SNK test with $\alpha = 0.05$. The data pertaining to mushroom yields were also given in the paper. Yields for each flush and total yield (overall two flushes), for each treatment, were expressed as kg per bag. After analysis of yield data, treatment means were compared and separated by the SNK test with $\alpha = 0.05$.

Results

Adult emergence. Adult emergence by treatment and sampling week are shown in Fig. 2. Treatment with water alone depicts emergence for uncontrolled mushroom production with the mean number of flies emerging increasing to over 1000 flies per m² in the 5th week of production. The weekly mean numbers of adult phorids that emerged from all treatments and the chlorpyrifos-ethyl-treated control were significantly lower than those in the water-treated control throughout the sampling period in the 3 successive growing periods (F = 84.27; df = 4, 445; P = 0.0001). Yet, there were no significant differences among the 3 microbial product treatments except for the *S. feltiae* treatment in the 2nd week (F = 1.51; df = 2, 268; P = 0.222), and these treatments did not differ significantly from the standard chlorpyrifos-ethyl treatment (F = 0.96; df = 3, 356; P = 0.439). When compared with the water control treatment, *Bti* reduced adult emergence by an overall mean of 76.9%, *S. feltiae* reduced emergence by an overall mean of 71.9%, and chlorpyrifos-ethyl reduced fly emergence by 72.4%.

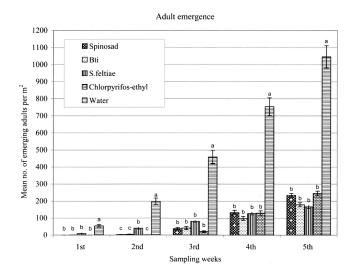


Fig. 2. Mean number (± SE) of emerging adult *Megaselia halterata* per m² in the treatments at each sampling week following treatment with spinosad, *Bti*, *S. feltiae* and chlorpyrifos-ethyl [Values are means of 3 growing periods, and means within a week followed by the same lower case letter are not significantly different (SNK, $P \le 0.05$)].

Larval damage. The spinosad, *Bti, S. feltiae*, and chlorpyrifos-ethyl treatments significantly reduced the incidence of larval damage to the mushrooms in comparison with the water-treated control treatment in each of the 3 growing periods (Table 1). Of the products tested, treatment with spinosad resulted in significantly lower damage than the 2 microbial products and the chlorpyrifos-ethyl in each growing season. Based upon the mean number of mushrooms damaged by the larvae, the order of efficacy of the materials tested changed only slightly with successive growing period. Order of efficacy, from highest to lowest, was spinosad > *Bti* = chlorpyrifos = *S. feltiae* (*F* = 4.27; df = 5, 24; *P* = 0.005) in the 1st growing period, spinosad > *Bti* = chlorpyrifos > *S. feltiae* (*F* = 3.59; df = 5, 24; *P* = 0.0001) in the 2nd growing period, and spinosad > *Bti* > *S. feltiae* = chlorpyrifos (*F* = 3.68; df = 5, 24; *P* = 0.0001) in the 3rd growing period. Incidence of fruit damage significantly changed among successive growing periods for *S. feltiae*, spinosad, and chlorpyrifos-ethyl, but not for *Bti* (Table 1).

There were 3 flushes of mushrooms over a 24-d harvesting period in each growing period; however the 3rd flush values were not taken into account in this paper due to disease outbreaks in some treatments. The yield for individual and cumulative flushes for each individual treatment is shown in Table 2. The 3 microbial product treatments and the water-treated control had significantly greater yield values for each flush and total yield (overall two flushes) than those subjected to the chlorpyrifos-ethyl treatment.

Discussion

Mushrooms are the most valuable protected crop grown in Turkey, and the only major horticultural commodity lacking an alternative to chemical control of pests. Our results suggest that microorganisms or their by-products can suppress mushroom phorid fly populations and reduce larval damage to the crop and may be viable alternatives to the conventional chemicals used in controlling these pests in Turkey.

Some biological alternatives to conventional insecticides used against dipteran pests in mushroom culture have been investigated. These include *Bti* and *S. feltiae*. Cantwell and Cantello (1984) and Keil et al. (1995a, b) reported that *Bti* was effective

Treatments	Growing Period I	Growing Period II	Growing Period III
Bacillus thuringiensis var. israelensis	15.0 ± 4.2Ab	14.2 ± 4.5Ab	14.8 ± 4.7Ab
Steinernema feltiae	17.0 ± 4.4Ab	21.3 ± 4.8Bc	19.8 ± 4.7ABc
Spinosad	8.0 ± 3.2Aa	7.2 ± 2.8.0Aa	11.3 ± 3.4Ba
Chlorpyrifos-ethyl	16.2 ± 5.8Ab	16.7 ± 6.7Ab	20.7 ± 6.9Bc
Water	43.0 ± 9.2Ac	49.3 ± 11.2Bd	55.5 ± 11.2Cd

Table 1. Mean percentage (± SE) of fruits damaged by the larvae of *Megaselia* halterata in each treatment and in 3 successive growing periods (GP)*

* Means within a row followed by the same capital letter are not significantly different (SNK, $P \le 0.05$) and means within a column followed by the same lower case letter are not significantly different (SNK, $P \le 0.05$).

Treatments	1 st flush yield	2 nd flush yield	Total yield
Bacillus thuringiensis var. israelensis	1.38 ± 0.3b	0.58 ± 0.2b	1.96 ± 0.4ab
Steinernema feltiae	1.56 ± 0.3a	0.71 ± 0.2a	2.27 ± 0.6a
Spinosad	1.36 ± 0.2b	$0.52 \pm 0.1c$	1.88 ± 0.5b
Chlorpyrifos-ethyl	0.76 ± 0.1d	0.28 ± 0.1e	1.04 ± 0.2d
Water	1.21 ± 0.3c	$0.43 \pm 0.2d$	$1.64 \pm 0.4c$

Table 2. Yield (kg per bag) obtained from the treatments tested in overall three growing periods (mean yield \pm SE)*

* Values are means of three growing periods, and means within a column followed by the same lower case letter are not significantly different (SNK, $P \le 0.05$).

in controlling some sciarid fly species in mushroom culture. We also found that Bti was effective against the mushroom phorid fly, M. halterata. The use of entomogenous nematodes, especially rhabditids of the genera Steinernema and Heterorhabditis, against mushroom flies (Richardson 1987, Nickle and Cantello 1991, Grewal et al. 1993, Scheepmaker et al. 1997, 1998) has been reported. For these nematodes, the mushroom bed environment is ideal with its warm moist conditions being maintained throughout the production cycle. The added advantage of nematodes over other biological controls is that their infective dauer stage larvae may actively seek the insect pest (Richardson 1987, Scheepmaker et al. 1998). Yet, Cantello et al. (1977) found that the steinernematid strain DD-136 did not parasitize and thereby control sciarids or phorids in mushroom culture. They suggested that the failure might be due to the nematodes migrating to the surface of the compost rather than remaining in the interior where the fly larvae were feeding. In contrast, Richardson (1983) found that both S. feltiae and Heterorhabditis heliothidis (Kahn, Brooks & Hirschmann) controlled cecids and sciarids. Richardson (1987) also determined that the larval stages of 3 dipteran species that commonly infest mushroom crops-M. halterata, Heteropeza pygmaea Winnertz (Cecidomyiidae), and Lycoriella auripila (Winnertz) (Sciaridae)were susceptible to parasitism by both S. feltiae and H. heliothidis. Our results demonstrated that the use of S. feltiae against M. halterata in mushroom culture is a viable alternative to conventional chemical insecticides, although Cantello et al. (1977) described unsuccessful attempts to parasitize phorids and sciarids with S. feltiae in compost-filled Petri dishes. Furthermore, entomophilic rhabditids of the genera Steinernema and Heterorhabditis are obligate parasites of insects in nature and have nonfeeding, free-living stages that do not affect plants or fungi and, thus, are safe for use in mushroom cultivation (Richardson 1987).

Little information is available on the effect of spinosad against mushroom flies. Spinosad reportedly provided poor sciarid fly control in casing and mixed results in compost (Anonymous 2004). In our experiments, we found that it was one of the most effective materials at controlling phorid fly populations. This difference in efficacy may be due to the use of different species from different families in these studies.

When taking into consideration yield values from the treatments, the lower values were obtained from the chlorpyrifos-ethyl treatment. This indicates that chlorpyrifos-ethyl treatments can cause mycotoxic side-effects, resulting in yield reductions. The

yield values also show that no microbial treatments had any adverse effect on mushroom formation and yield when compared with the water-treated control.

In conclusion, our results suggest that 3 microbial (by-) products, *Bti*, spinosad and *S. feltiae*, may become viable alternatives to chemicals because they appear as effective as chlorpyrifos-ethyl in reducing fly emergence and larval damage to the mushroom crop. The use of these products in conjunction with good management (in the form of compost pasteurization, fly screening, fumigation of rooms, and general good hygiene) can reduce the use of chemical insecticides, or enable insecticide-free production, and may provide a marketing benefit.

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