CHEMICAL AND BIOLOGICAL CONTROL OF DIPTERA IN SPANISH MUSHROOM CROPS

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ABSTRACT

The phorid *Megaselia halterata* and the sciarid *Lycoriella auripila* are common pests in Spanish mushroom farms. *M. halterata* has been seen to be the dominant species (ratio 4:1), with the highest number in spring and autumn. The objective of this study was to look for alternatives to control mushroom flies, especially phorids. Several assays were carried out using entomopathogenic nematodes [106 IJ m^{-2} *Steinernema feltiae* and 0.5 x 10^6 IJ m^{-2} *S. feltiae* + 0.5 x 10^6 IJ m^{-2} *S. carpocapsae*], and the insecticides diflubenzuron [1 g m^{-2}] and triflumuron [0.5 and 1 g m^{-2}]. The effectiveness against flies and effect on yield were evaluated. Effectiveness trials were set up in an experimental growing room, after spawn running trays were exposed to diptera infestation in a commercial crop. On days 16-17, after casing, treatments were applied. The experiments terminated after the emergence of the first generation of adults. The captured flies were identified and the percentage reduction in adult emergence was calculated. Phytotoxic trials were set up as described above, but without infestation. The effect of treatments on productivity was evaluated from the yield, the biological efficiency of the crop and the earliness of the harvest. No decrease in the population of *M. halterata* was detected with either nematode or insecticide treatment. Population of *L. auripila* fell with both nematode treatments applied, particularly with the *S. feltiae* treatment. Diflubenzuron and triflumuron were highly effective against sciarids, regardless of the application dose. The production parameters were not affected by any treatment.

Keywords: *Megaselia halterata*, *Lycoriella auripila*, diflubenzuron, triflumuron, entomopathogenic nematodes

INTRODUCTION

The phorid *Megaselia halterata* (Wood) and the sciarid *Lycoriella auripila* Winnertz are common pests in mushroom farms of Castilla-La Mancha, Spain [1]. In their larval stage, these flies feed on mushroom mycelia and even burrow into mushrooms once they are formed [2]; as adults, they act as vectors of other pests and diseases [2-5]. Unlike in other European countries, where sciarids are present throughout the year and phorids undergo a period of hibernation [6], in Spain *M. halterata* populations are present in high numbers throughout the year, whereas *L. auripila* is detected in much lower numbers (four times less than *M. halterata*) and mainly in spring and autumn [1]. Traditionally, flies in mushroom crops have been controlled by insecticides. Other methods for controlling flies are based on the use of physical barriers to exclude adult individuals from growing in farms [7], by biocontrol organisms, such as mites, bacteria and entomopathogenic nematodes [8-11]; and, more recently, by plant extracts [12-16].

In the last years of twenty century, the list of insecticides allowed for using in European mushroom farms was very large: malathion, diazinon, methoprene, permetrin, dichlorvos, chlorpyrifos, deltametrin, carbofuran, bendiocarb, cyromazine, diflubenzuron, triflumuron and azadirachtin. Literature shows varying results with regards to the efficacy against phorids and/or sciarids [15,17-21]. However, the application of phytosanitary products may give rise to two further problems: detrimental effects on the mushroom mycelium, leading to a loss of yield or quality [2,15,19, 22-25], and the presence of residues in the mushrooms once harvested [26]. Furthermore, resistance to certain products has been recorded [9, 27-28]. It must also be borne in mind that the evaluation of active substances set in motion by the EU (Directive 91/414/ECC) has considerably reduced the number of insecticides authorized for use in mushroom cultivation: at this moment, just malathion, chlorpyrifos, deltametrin, cyromazine, diflubenzuron, triflumuron and azadirachtin are allowed in Europe mushroom farms. In Spain, deltametrin (2.5%, [EC] P/V, 1.5% [EW] P/V), azadirachtin (3.2%, [EC] P/V), diflubenzuron (25%, WP) and cyromazine (75%, [WP] P/P) are the only insecticides allowed for use in mushroom crops. Deltametrin and azadirachtin are effective against adult flies, whereas diflubenzuron and cyromazine are effective against sciarid larvaes

[2,14, 29-31], but not against phorids [18,32]. Spanish mushroom growers have not any tool for controlling phorid populations [30]. Triflumuron has been shown effective against sciarids [5, 31], but information on the effectiveness against phorid has not been found.

As regards the biocontrol organisms, nematodes are often applied to control mushroom flies. Richardson [33] was the first to use entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* to control mushroom flies [33]. *Steinernema* spp. locate and invade fly larvae via anus, mouth or spiracles, and release bacteria associated with them (*Xenorhabdus* sp. or *Photorhabdus* sp.), which provokes the death of the infested flies [34]. However, apart from their compatibility with the insecticides [35], the effectiveness of nematodes in mushroom culture depend on other factors such as temperature, moisture and CO_2 levels, which may also affect them [34]. Information about phorid control by nematodes is scarce in the literature. The application of *S. feltiae* against phorid flies has shown varied results [9,16,32], whereas *S. carpocapsae* treatments have got favourable results [11]. For the control of sciarids, many authors recommend the use of *S. feltiae* [9,14]. In the case of *S.carpocapsae*, Gouge and Hague [36] reported the relative inefficacy of this species against sciarids, whereas, other authors defend its efficacy, but with a lower infectivity than that of *S. feltiae* [37]. Furthermore, some authors observed a detrimental effect of treatments involving entomophathogenic nematodes on mushroom mycelia, such as reduced yield in early flushes, depending on the nematode dosage rate [24].

The objective of this study was to look for alternatives to control mushroom flies, especially phorids, in Spanish mushroom farms checking insecticides as triflumuron, and entomopathogenic nematodes as *S. feltiae* and *S. carpocapsae*.

MATERIALS AND METHODS

Effectiveness assays

Effectiveness trials were set up in an experimental mushroom growing room. At the end of the spawn running, some trays (10 kg of compost, 870 cm^2 per tray) were exposed to infestation in a commercial mushroom farm, where they were left for 48 hours. Afterwards, the trays were placed in the experimental room again, where they were covered with a structure made of anti-trips mesh. A yellow sticky trap was placed inside each of them to capture the flies. On days 16-17, after casing, treatments were applied.

Two nematode effectiveness trials were set up. A randomised complete block design with four treatments and ten replicates was used in the nematode trials: Sf, *S. feltiae* treatment, fly-infested trays and subsequent application of 10^6 IJ m^{-2} *S. feltiae*, Biorend R champiñones, (Idebio S.L., Salamanca, Spain) together with 1 ml of chitosan in 150 ml water per tray; Sf+Sc, *S. feltiae*+*S. carpocapsae* treatment, infested trays and subsequent application of $0.5x10^6$ IJ m^{-2} *S. feltiae*+ $0.5x10^6$ IJ m^{-2} *S. carpocapsae*, Biorend R champiñones + Biorend Rpalmeras, (Idebio S.L., Salamanca, Spain) together with 1 ml of chitosan in 150 ml water per tray. Ten trays free of fly infestation and with no nematode application were used as non-infested controls (C), and ten fly-infested trays with no nematode application were used as infested controls (IC). In the first trial, trays were exposed to fly infection pressure of 800 phorids and 52 sciarids per day on day 5 of spawn running. In the second trial, on day 11 of spawn running trays were exposed to infection of 1,237 phorids and 82 sciarids per day.

Two insecticide effectiveness trials were set up. A randomised complete block design with four treatments and six replicates was used in the insecticide trials: IC, fly-infested trays; *D*, fly-infested trays and subsequent application of diflubenzuron (Dimilin, UniRoyal Chemical) (1 g m^{-2} a.i.); *A1*, fly-infested trays and subsequent application of triflumuron (Alsystin, Bayer Crop Science SL) (0.5 g m^{-2}a.i.); *A2*, fly-infested trays and subsequent application of triflumuron (1 g m^{-2}a.i.).Trays were exposed tofly infection on day 11 of spawn running. The infection fly pressure was 584 phorids and 139 sciarids per day in the first trial, and 388 phorids and 29 sciarids per day in the second trial. The experiments finished after the emergence of the first generation of adults. The captured flies were counted and identified and the percentage reduction in adult emergence was calculated.

Effect on mushroom production

Nematode trial was set up in the same way as described before, but there was no infestation. A randomised complete block design with three treatments and twelve replicates (10 kg of compost, 870 cm^2 per replicate) was used in the trials. Treatments were C, Sf and Sf + Sc. Mushrooms were harvested daily for two flushes. The number and total weight of the fruit bodies were recorded for each treatment. The effect of entomopathogenic nematodes on mushroom productivity was evaluated from the total yield of harvested mushrooms, and from the number and unitary weight of mushrooms. The effect of treatments on mushroom productivity was also evaluated from the biological efficiency of the crop, calculated as the ratio of the fresh weight of the total yield of harvested mushrooms to the weight of dry substrate at spawning, expressing the fraction as kg $100kg^{-1}$ compost. In addition, the earliness of each treatment was expressed as the number of days between casing and harvesting of the first flush.

A randomised complete block design with four treatments and six replicates (84 kg of compost, 1.5 m^2 per replicate) was used in the insecticide trial. Treatments were: *C*, control trays, and *D*, *A1* and *A2* treatments. Mushrooms were harvested daily for three flushes.The effect of insecticides on mushroom productivity was evaluated from the total yield of harvested mushrooms. Biological efficiency and the earliness were also calculated.

Statistical Analysis

Analysis of variance, with logarithmic or square root transformation for count data and angular transformation of the percentage data to stabilize variances when necessary, were done using the software package Statgraphics Plus, version 4.1 (StatisticalGraphics Corp., Princeton, NJ, USA). Tukey's test, at 5% probability, was used to establish significant differences between means.

RESULTS AND DISCUSSION

Nematode effectiveness assays

The total number of flies captured in the first trial was lower than in the second one, reflecting the lower degree of initial infection pressure in the first trial. Although fly captures were registered in the non-infested trays C (Table 1), in both trials and for both flies, the data obtained for treatment IC were significantly higher than those for C, indicating the good progress of the infection process. In the first trial, the mean number of emerging *M. halterata* was 233 for the infested control trays (IC), and 186.3 and 221.8 for the nematode treatments Sf and Sf+Sc, respectively, with no significant differences between them ($F_{2,29} = 0.63$; $p = 0.5415$). No significant differences ($F_{2,23} = 1.21$; $p = 0.3168$) were observed between the numbers of phorid flies captured in the treatments IC, Sf, and Sf+Sc in the second trial (Table 1). There was also no effect of the treatments Sf and Sf+Sc in the emergence of *M. halterata* adults in both trials, and no phorid percentage reduction was verified for any treatment.

Table 1. Total number (mean and standard deviation) of phorids and sciarids captured for each of the nematode treatments in both assays. In the case of *L. auripila* the percentage reduction obtained for each treatment in both trials is also included[(1)].

Treatments	*M. halterata*		*L. auripila*		*L. auripila* reduction (%)[(2)]	
	First trial	Second trial	First trial	Second trial	First trial	Second trial
C*	14.1±6.9a	22.3±18.0a	2.0±2.2a	40.7±13.2a	—	—
IC	233.0±97.6b	462.7±166.2b	136.2±60.7c	184.4±46.7c	—	—
Sf	186.3±57.3b	366.1±80.3b	50.3±26.2b	116.2±35.4b	63.1B	37.0A
Sf+Sc	221.8±121.6b	406.3±133.2b	68.1±47.4b	180.0±53.2c	50.0B	2.4A

[(1)]For each trial, means followed by equal letters, lowercase in the columns, do not differ significantly by Tukey's test, at 5% probability.
[(2)]For reduction (%), means followed by equal uppercase letters in the files do not differ significantly by Tukey's test, at 5% probability.
*C, non-infested control; IC, infested control without nematode treatments; Sf, nematode application of 10^6IJ m^{-2}*Steinernema feltiae*; Sf+Sc, nematode application of $0.5x10^6$ IJ m^{-2} *S. feltiae* + $0.5x10^6$IJ m^{-2} *S. carpocapsae.*

The lack of effectiveness of *S. feltiae* for controlling phorids agrees with most of the consulted literature [32,38]. However, it was not in agreement the findings of Long *et al*. [39] in laboratory bioassays. These authors reported that *S. feltiae*is effective in the control of *M. halterata*, probably because nematodes applied on growing substrates show different behaviour. The results of the present work also differed with the findings of other authors, who observed a reduction of more than 70% in the emergence of *M. halterata* adults after the application of *S. feltiae* [9,16]. This divergence in results was probably due to the fact that in the present study only the first generation of adults after infestation was considered, whereas the above authors also considered subsequent generations. The ineffectiveness of the Sf+Sc treatment on *M. halterata* was observed regardless of the trials, which differed with the results obtained by Jess and Bingham [11], who confirmed the use of *S. carpocapsae* to control phorids. This discrepancy might be due to the lower application doses used or to the application of both nematode species. In trial conditions, the inefficacy of both treatments against *M. halterata* was independent of the time elapsing between infestation and nematode application. For optimum efficacy, nematodes should be applied when a majority of hosts are susceptibleto nematode infection. In the first trial, considering the environmental conditions and the time elapsing between infestation and treatment (9–10 days), the larvae were presumed to be in a sufficiently advanced stage (third larval instar) to allow infestation by nematodes [11, 38]. However, not even in these favorable conditions did the applications lead to an acceptable level of reduction when the nematode formulations were used. Further efforts to develop entomopathogenic nematodes against *M. halterata* are necessary.

As regards the sciarid flies, in the first trial, the mean number of emerging *L. auripila* was 136 for the infested control trays (IC), and significantly lower ($F_{2,29}$= 9.42; p = 0.0007) for both nematode treatments. There was no significant difference between the Sf and Sf+Sc treatments (Table 1). In the second trial, 184 sciarid flies were captured emerging from the infested control treatment, a value statistically similar to that recorded for the Sf+Sc treatment; however, this number was significantly reduced ($F_{2,23}$ = 6.53; p = 0.0057) in the Sf treatment (116 sciarid flies). In this trial, only the Sf treatment reduced the adult emergence of *L. auripila* when compared to the infested control. Gouge and Hague [3] established that *S. carpocapsae* was relatively ineffective against different sciarid species. The results obtained in the present work are in agreement with this, but contradict those of Kim *et al*. [37], who found that *S. carpocapsae* can be an effective tool for the management of the fungus gnat larvae [37]. Both nematode species were applied together so that each could combat its own target pest. However, there was no improvement in the efficacy of the combined treatment against either fly species targeted. For the control of sciarids (Table 1), the reduction percentage (63%) obtained with the Sf treatment in the first trial can be considered satisfactory; however, it was less than the 80–95% reduction in sciarids obtained by other authors [9,14, 40]. In the second trial, the reduction percentage (37%) was significantly lower than that in the first trial ($F_{1,18}$ = 9.15; p = 0.0073). In the case of the Sf+Sc treatment, the reduction percentage obtained in first trial (50%) was also satisfactory and significantly higher than the one in the second trial ($F_{1,13}$ = 19.41; p = 0.0007). Efficacy was lower for each treatment in the second trial. Considering the environmental conditions and the time elapsing between infestation and treatment (3–4 days), larvae were probably not large enough to be parasitized. This agrees with the recommendation of Scheepmaker *et al*. [9] to postpone treatment to a week after infestation. A longer time between infestation and nematode application would favour fly control because the nematodes would find larger larvae (third and fourth stage), which are more vulnerable to *S. feltiae* attack [11,38].

Insecticide effectiveness assays

In the first trial, the mean number of emerging *M. halterata* was 512.7 for the infested control trays (*IC*), and 577.8, 503.8 and 388.5 for the insecticide treatments *D, A1* and *A2*, respectively, with no significant differences between them ($F_{3,20}$ = 0.31; p = 0.8213) (Table 2). In the second trial, no significant differences between the mean number of emerging *M. halterata* captured in all of the treatments ($F_{3,20}$ = 0.91; p = 0.4525) (Table 2) were registered. No phorid percentage reduction was verified for any treatment.

As regards, *L. auripila*, in the first trial, the mean value of captures for the infested control trays (*IC*) was significantly higher ($F_{3,20}$ = 87.99; p = 0.0000) than those for the insecticide treatments (*A1, A2* and *D*), which noted a 99% sciarid emergence reduction (Table 2). In the second trial, significant differences between number of sciarid captured for *IC* and insecticide treatment *D, A1* and *A2* were also noted ($F_{3,19}$ = 92.23; p = 0.0000), with 97% of emergence reduction (Table 2).

Table 2. Total number (mean and standard deviation) of phorids and sciarids captured for each of the insecticide treatments in both assays[(1)].

Treatments	*M. halterata*		*L. auripila*	
	First trial	Second trial	First trial	Second trial
*IC**	512.7 ± 219.3	666.7 ± 365.7	649.8 ± 137.5b	143.5 ± 91.0b
D	577.8 ± 437.8	915.2 ± 986.3	4.0 ± 5.0a	2.4 ± 1.3a
A1	503.8 ± 476.6	846.7 ± 571.5	5.0 ± 4.1a	2.3 ± 2.0a
A2	388.5 ± 143.2	1,110.0 ± 439.2	2.3 ± 1.5a	2.7 ± 0.8a

[(1)]For each trial, means followed by equal letters, lower case in the columns, do not differ significantly by Tukey's test, at 5% probability. **IC*, infested control without insecticide treatments; *D*, fly-infested trays and subsequent application of diflubenzuron (1 g m^{-2}a.i.); *A1*, fly-infested trays and subsequent application of triflumuron (0.5 g m^{-2}a.i.); *A2*, fly-infested trays and subsequent application of triflumuron (1 g m^{-2}a.i.).

As well as diflubenzuron [30,32], the application of triflumuron had not any effect on the *M. halterata* populations, regardless of the infestation pressure and/or the rate. Up to now, authors are ignorant of any reference about the effectiveness of triflumuron on mushroom phorids. On the other hand, the application of triflumuron was very effective against *L. auripila* populations, regardless of the infestation pressure and/or the rate. This result agrees with Shamshad *et al.* [5], who described an excellent effectiveness of triflumuron 25% WP (application rate: 20 mg kg^{-1}) on *Bradysia ocellaris* (Comstock) populations in Australia. Erler *et al.* [31] also described 78% of *L. ingenue* (Dufour) population reduction as results of triflumuron 48% application (1g m^{-2}) in Turkey.

Effect of nematode treatments on mushroom production

The mean values of the mushroom yields, per flush and total yield, for each of the treatments (C, Sf, and Sf+Sc) were statistically similar for the first flush ($F_{2,31}$ = 1.60; p = 0.2188), the second flush ($F_{2,31}$ = 2.98; p = 0.0657), and for total harvest ($F_{2,31}$ = 0.31; p = 0.7346); a total yield of around 20 kg m^{-2} was obtained in all three cases (Table 3). This result reflects the statistically similar biological efficiency ($F_{2,31}$ = 0.31; p = 0.7343) of all the treatments, with values close to 85 kg of mushrooms per 100 kg of compost (dry weight) collected over two flushes (Table 3).

Regarding the number of mushrooms harvested, both treatments showed lower numbers than the control in the first flush. In the Sf+Sc treatment this decrease was compensated in the second flush since the total results for total yield only showed a significant drop ($F_{2,31}$ = 5.28; p = 0.0107) for the Sf treatment (Table 3). Moreover, the unitary weight of the mushrooms was significantly higher ($F_{2,31}$= 10.27; p = 0.0001) for the Sf treatment than for the control and for Sf+Sc (Table 3). Lastly, earliness, defined as the time between applying the casing mixture and the first flush, varied between 21.5 and 21.7 days, with no significant differences ($F_{2,31}$ = 0.28; p = 0.7577) between treatments.

Table 3. Mushroom production (kg m^{-2} and number m^{-2}), Biological Efficiency (BE: kg of mushroom 100 kg^{-1} dried compost), Unitary weight (g) and Earliness (days to first flush harvest) in each of the treatments[(1)].

Treatments	First flush		Second Flush		Total harvest		BE	Unitary weight	Earliness
	Yield	Nº	Yield	Nº	Yield	Nº			
C*	12.1	963b	7.6	639ab	19.7	1602b	84.8	12.5a	21.5
Sf	11.1	708a	8.7	588a	19.8	1296a	85.3	15.4b	21.6
Sf + Sc	11.6	776a	7.7	783b	19.3	1559b	83.0	12.7a	21.7

[(1)]Means within a column followed by the same letter are not significantly different according to Tukey's test (p<0.05).
*C: control; Sf: trays with nematode application (10^6 IJ m^{-2} of *S. feltiae*); Sf+Sc: trays with nematode application (0.5 x 10^6 + 0.5 x 10^6) IJ m^{-2} of (*S. feltiae* + *S. carpocapsae*).

Some authors maintain that the use of entomopathogenic nematodes affects mycelial growth, depending on the nematode dosage rate [24], leading to decreased yields for the first flush,although subsequent flushes typically compensate for this early yield loss [38]. The results found in the present work contradict this affirmation since the overall yields were similar in both flushes for all treatments. For the number of carpophores collected, Grewal *et al.* [24] obtained 20% more mushrooms after the application of *S. feltiae*, which was attributed to the increased dispersion of the bacterium *Pseudomonas putida*, responsible for the formation of primordia in the casing material. In the present study,the application of *S. feltiae* alone reduced the number of mushrooms harvested, but the unitary weight was higher, which might be considered advantageous for post-harvest quality management, especially in the first flush when there are normally too many mushrooms to pick [25].

Effect of insecticide treatments on mushroom production

There were no statistical differences of yield values between the treatments (Table 4) for first flush ($F_{3,20}$= 0.26; p = 0.8520), second ($F_{3,20}$ = 0.15; p = 0.9266), and third flush ($F_{3,20}$= 1.03; p = 0.3996), neither for total harvest ($F_{3,20}$= 0.11; p = 0.9512). Insecticide application had not any effect on biological efficiency ($F_{3,20}$= 0.11; p = 0.9512) neither on earliness ($F_{3,19}$= 0.76; p = 0.5304), with values of 83.43-85 kg mushroom per 100 kg dried compost and 23.7-23.8 days, respectively for all treatments (Tabla 4).

Table 4. Yield (kg m^{-2}) of mushroom crop, biological efficiency and earliness (days)[(1)]

Treatments	First Flush	Second Flush	Third Flush	Total Harvest	BE	Earliness
*C**	6.5	10.8	3.5	20.9	83.4	23.7
D	6.3	10.4	4.2	20.9	83.7	23.8
A1	6.4	10.7	4.1	21.2	85.0	23.7
A2	6.2	10.5	4.1	20.8	83.3	23.8

[(1)]Means within a column followed by the same letter are not significantly different according to Tukey's test (p<0.05).
**C*: control without insecticide treatments; *D*, application of diflubenzuron (1 g m^{-2}a.i.); *A1*, application of triflumuron (0.5 g m^{-2}a.i.); *A2*, application of triflumuron (1 g m^{-2}a.i.).

The results shown that triflumuron has not any phytotoxic effect on mushroom production. This affirmation agrees with Shamshad *et al.* [5] and Erler *et al.* [31] neither of whom described production reduction attached application of triflumuron on casing.

CONCLUSION

The application of *Steinernema feltiae* ten days after the foreseen time of infestation is beneficial for the control of sciarids.Triflumuron and diflubenzuron are also effective for the control of mushroom sciarids.The application of diflubenzuron, triflumuron or entomopathogenic nematodes has no effect on the phorids found in mushroom crops.The application of insecticides diflubenzuron or triflumuron, or the application of entomopathogenic nematodes has no adverse effect on mushroom production.

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