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Article

Prey preference pattern of *Gaeolaelaps aculeifer* (Acari: Laelapidae) on fungus gnats, *Lycoriella auripila* (Diptera: Sciaridae); a step toward efficient release

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Abstract

Lycoriella auripila Winnertz (Diptera: Sciaridae) is one of the most serious pests of mushroom cultures, *Agaricus bisporus* (Lange) Imbach. Damage of this pest can result in substantial yield losses directly and indirectly. *Gaeolaelaps aculeifer* Canestrini as a predator suppresses population of soil-dwelling pests e.g. mites, nematodes and fungi, and todays it has been considered as a commercial biological control agent against eggs and larvae of sciarid flies. Prey preference of natural enemies is one of the components of predation that should be taken into account to choose the exact releasing time against appropriate prey stages. In this study, the prey preference and predation rate of *G. aculeifer* were investigated at 23±1 °C, 60 ± 5% R.H. and a photoperiod of 0: 24 (L: D) h under laboratory conditions. Results showed that life stages of prey influenced prey preference, as females of *G. aculeifer* killed 5.66 ± 0.33 and 2.33 ± 0.21 of 2nd and 3rd instar larvae of *L. auripila* respectively. Accordingly, Manly's index of preference was 0.697 ± 0.0377 and 0.303 ± 0.0377, respectively. This difference might be attributed to prey size difference between 2nd (1.36 ± 0.08 mm) and 3rd (2.52 ± 0.10mm) sciarid larval instars. Moreover, in terms of obtained results, proper timing of releasing predatory mite reduces sciarid fly population efficiently. It has been recommended that releasing of *G. aculeifer* females prior to emergence of 3rd instar larvae of *L. auripila* would suppress the sciarid flies population densities in mushroom production.

Key words: *Agaricus bisporus*; biocontrol; Manly's index; predation rate; sciarid fly.

Introduction

Nowadays, the growing mushroom is an industrial and profitable profession worldwide. Edible mushrooms are rich food sources including proteins, unsaturated fatty acids, fibers and vitamins. A large area has been allocated to cultivate button mushrooms whose most common species is *Agaricus bisporus* Lange (Mehrparvar *et al*. 2014).

Different pest species attack commercial productions of common mushroom. Among them, sciarid flies (Diptrae: Sciaridae) have the highest importance and can be very destructive. Several genera from this family infest edible mushrooms, which the most important of them are *Lycoriella* spp. These are small flies, body elongated and are about

3–4 mm with long legs and membranous wings, although their most important feature is the presence of vein Y (Fletcher *et al.* 2008).

Sciarids go through seven life stages including, egg, four larval instars, pupae and adults. Females mostly tend to oviposit into the soil from seeding until the sixth day of spawn-running off seeds. Female fecundity ranges from 100 to 200 eggs laid at 1.9–3.8 cm of compost depth. If larvae attack the primordia, reproductive organs or sporophore will not be formed, thus larval damage is notable, sometimes causes the yield loss up to the 90 percent (Shirvani-Farsani 2011). Infective larvae attack reproductive organs and enter mushroom bodies from its base and create a brown canal. In addition to direct damage, the larvae transmit various pathogenic microorganisms to mushrooms causing breakage, discoloration and decay of outer tissues. Furthermore, larval feces of sciarids cause the diminishing physical and chemical properties of compost, which in turn, declines the marketing value of the product. The adults do not feed, but they can transmit various disease agents such as *Verticillium fungicola* Preuss, 1851 (Dry Bubble Disease) and *Pseudomonas tolaasii* Paine, 1919 (Dry Bubble or Brown Blotch Disease) and decrease the quality of mushrooms (Fletcher *et al.* 2008).

Referring to low cost of Organochlorine, Organophosphate or Benzoin compounds and simple mode of application, frequent use of these pesticides on edible mushrooms was a main challenge for consumers. It was observed that besides to the mycotoxic effects, some other problems also may arise with the frequent use of chemical-based control strategies, including pest resistance to pesticides and environmental issues (Jess and Bingham 2004).

Biological control is as an environmental friendly tactic against insect pests and could be considered as an alternative for chemical control and other traditional control methods in plant protection (Frumkin 2010).

Laelapid mites (order Mesostigmata) constitute a large family with worldwide distribution. Many of them are external parasites of small mammals, some others are free living predators which feed on different terrestrial preys such as mushroom sciarid larvae and nematodes (Lindquist *et al*. 2009). One of the well-known species of this family is *Geaolealaps aculeifer* (Canestrini, 1884) [formerly known as *Hypoaspis aculeifer*] (Acari: Laelapidae) (Jess and Bingham 2004). Entomite-A, the commercially products of *G. aculifer*, has been recommended against soil-dwelling stages of sciarid flies, smaller larvae preferentially and bulb mites in lily (Koppert Biological System 2016).

The successful implementation of biological control programs depends on various factors. A critical feature of predators in each biocontrol program is the prey preference, which should be studied precisely. Otherwise, there is a risk that unsuitable prey stage (stages) is selected for releasing and the required synchronization of biocontrol agents with pest vulnerable stages being lost (Ceballos *et al*. 2009; Alaee and Allahyari 2013).

Determining the proper time to release natural enemies is of critical importance in each biocontrol project success and ignoring the prey preference of natural enemies might lead to failure in biocontrol projects. Therefore, investigating the food preference of biological control agents is of great importance.

The aim of this study was to investigate prey preference and predation rate of *G. aculeifer* on second and third instar larvae of *L. auripila*. According to the importance of mushroom production as a household job and industrial productions, this study could be a first step toward implementing and determining the optimal releasing time of *G. aculeifer* against sciarid flies. Additionally, the results of releasing *G. aculeifer* against preferred stage of sciarid flies on mushroom compost was evaluated in a mushroom production workhouse.

Materials and methods

Insect culture

Adults *L. auripila* were collected from commercial mushroom cultures (Hamedan province, February to June 2015). All samples were brought back to the laboratory and introduced into the net covered cages (12 × 12 × 18 cm) containing mushroom compost. Many eggs and larvae could be collected from the surface of the peat in each cage.

Adults of *G. aculeifer* were originally taken from Iranian Research Institute of Plant Protection and were reared under laboratory conditions in plastic containers (10 × 6 × 4 cm). Potato slices were used as oviposition substrate.

Both predator and prey cultures were kept at the climate growth chamber (23±1 °C, 60 ± 5% R.H. and a photoperiod of 0: 24 (L: D) h).

No-choice and choice tests

Experiments were performed in plastic containers (7 × 4 × 3 cm). To supply the humidity, foam containers were covered with a mixture of plaster of Paris and charcoal (with the ratio of 7 to 1) and water was sprinkled every other day.

In order to investigate predation rate of *G. aculeifer* on 2nd, 3rd and 4th instars larvae of *L. auripila*, 15 *L. auripila* larvae of any of the those stages were offered to *G. aculeifer* females individually in a no-choice test. Then, after 24 hours the number of killed larvae was counted. To determine the initial number of prey offered in a choice test, usually 70% of maximum predation rate was considered. Thus, 10 individuals from second, third and fourth instar larvae of *L. auripila* were used in choice test of prey preference of *G. aculeifer*. After 24 hours, the number of survived larvae was recorded in each container. Control treatment without predator was checked for corrected mortality using Abbott's formula. All treatments were replicated 15 times.

Releasing predatory mite at a mushroom production workhouse scale

Mushroom composts were prepared from Sahar mushroom company brought back to the mushroom production workhouse (Hamedan province, Bahar city). Composts were transferred into plastic baskets (30 × 42 × 10 cm) covered by net cages (30 × 42 × 60 cm) placed at 23 ± 2 °C, 75 ± 5% R.H. and photoperiod of 0: 24 (L: D) h. After spawn-running typically took two weeks, casing layer was done for prompting mushroom production. The peat soil was applied for casing in 4–5 cm depth and thermal shock was performed at this stage (temperature reduced to 18 °C). Then, 50 pairs of male and female sciarid flies were released into each cage to infect compost. These flies were all removed by sticky traps after 3 days. Then, three treatments including release of 100 *G. aculeifer* immediately and 12 days later (against older sciarid larvae) plus control (without predator release) were applied. Finally, after 15 days of release of sciarids, the emergence of next generation of pest was monitored by yellow sticky traps. Treatments were repeated 10 times at 18 ± 1 °C, 75 ± 5% R.H. and 24:0 (L: D).

Data analysis

Choice test experiment

Different indices have been used to study the preference of the predators (Cock

1978). Among them, only Manly's β index (Manly 1974) allows prey exploitation i.e. it considers the decreasing density of prey during the experiment period and there is no need to prey replacement). It is clear that in the absence of any preference, the changes will be as a ratio of primary density.

Manly's index of preference could be calculated as:

$$B_i = \frac{\log(\frac{e_i}{A_i})}{\sum_{s=1}^{k} \log(\frac{e_s}{A_s})}$$

Where, β_i is the predator preference index to prey type i, e_i is the number of survived prey of prey type i, A_i is initial number of prey belongs to type i, e_s is total number of survived prey belongs to the prey type s and A_s is the total initial number of prey types. The symbols s and k shows the number of different prey types. If $\beta = 0.5$, the predator does not show any preference in relation to prey types. Preference indices were statistically analyzed using paired t-test.

Referred to the morphometric characteristics, body length and weight of sciarid fly larvae were measured using a caliper and digital scale (0000).

Releasing G. aculeifer

Data normality assessed by Shapiro-Wilcoxon test. Then, one-way ANOVA were used to determine the effect of predatory mite treatments on *L. auripila* population. Tukey's multiple range tests was used to compare mean values and ranking of treatments.

Results and Discussion

The results showed that prey life stage affects the predation rate and prey preference, as the predation rate of seven-day females of *G. aculeifer* on second and third larval stages of *L. auripila* was recorded as 5.66 ± 0.33 and 2.33 ± 0.21, respectively (Table 1) which was significantly different ($t = 11.979$, $df = 14$, P-value = 0.0001). It was found that *G. aculeifer* was not able to feed on *L. auripila* 4th instar larvae, so preference study was just carried out with second and third instar larvae of sciarid instead.

Accordingly, preference index of *G. aculeifer* toward second and third instar larvae of *L. auripila* was significantly different ($t = 5.213$, $df = 14$, P-value = 0.0001) (Table 1).

Table 1. Prey preference index (mean ± SE) and predation rate of *Gaeolaelaps aculeifer* on 2nd and 3rd instars larvae of *Lycoriella auripila*.

	2nd instar larvae	3rd instar larvae
Manly's index (β)	0.697 ± 0.0377*	0.303 ± 0.0377
Predation rate	5.66 ± 0.33	2.33 ± 0.21

*Significantly different at 5% level.

Several factors can be influential on prey preference of *G. aculeifer* of sciarid fly larvae. More importantly, the observed difference in preference index and the predation rate of *G. aculeifer* could be attributed to different size of second to fourth larval instars of sciarids (Table 2). *Gaeolaelaps aculeifer* females are 0.8–0.9 mm in length (OECD 2008), therefore with regard to the obvious differences in the size of three and four instar larvae of *L. auripila* with the predatory mite females, subdue and bring the larger prey under control will be big difficult. This issue has been addressed in other researches such as Salvucci and Nedved (2008), who noted the physical structure and body size of prey are also effective in accepting certain prey.

The second important elements of different preference might be the defensive mechanisms and reactions of prey species against predator. Defensive reaction is size dependent characteristic and it is expected that larger preys defends themselves more efficiently.

Table 2. Body weight (mean ± SE) (mg) and length (mean ± SE) (mm) for developmental stages of *Lycoriella auripila*.

Parameter	2nd instar larva	3rd instar larva	4th instar larva
Body weight	0.0477 ± 0.0031	0.1392 ± 0.0032	0.8371 ± 0.0242
Body length	1.36± 0.08	2.52 ± 0.10	4.91 ± 0.19

The results of releasing *G. aculeifer* showed that the presence of predatory mite significantly decreased sciarid flies populations relative to control treatments (F = 33.896, df = 2, p-value < 0.0001). The releasing of *G. aculeifer* immediately after removing *L. auripila* adults (as a preventive treatment) was more efficient than treatments (Table 3). Our results confirmed the impact of the exact releasing time of natural enemies on final population of this pest.

Table 3. Number of *Lycoriella auripila* caught on yellow sticky traps.

Treatments	Preventive release	Delayed release	Control
Number of emerged pest (Mean ± SE)	173.5 ± 10.75 c	354.1 ± 22.54 b	519± 44.92a

Note: Different letter in the columns are significantly different at p < 0.01.

Gaeolaelaps aculeifer encounters with a wide prey range of different species inside soils, however it has been suggested that the efficiency of this predator against various prey types is not the same (Brødsgaard *et al.* 1996).

There are a few studies on predation rate of *G. aculeifer* on sciarid flies and little information were available on the predation rate or preference of *G. aculeifer* by feeding on different stages of sciarids. Instead, several studies were conducted on similar species such as *Hypoaspis miles* Berlese, 1892 using different prey species.

In one of the most pertinent studies, Enkegaard *et al*. (1997) reported that the average daily consumption of immature and adult stages of *H. miles* feeding on larvae of *Lycoriella solani* Winnertz, 1871 (Diptera: Sciaridae) and *Tyrophagus putrescentiae* (Schrank, 1781) (Acari: Acaridae) which were 0.24–0.86 and 10.8–21.7, respectively. In another study it has been shown that *H. aculeifer* preferred larval stage of *Rhizoglyphus echinopus* Fumouze and Robin, 1868 (Acari: Acaridae) in comparison with other stages (Ragusa and Zedan 1988). Such that female of *H. aculeifer* consumed 193.8 ± 44.6 *R. echinopus* larvae stages, during their immature stages. Additionally, young stages of *H. aculeifer* didn't have any preference for tritonymph and adult stages of this prey. Daily predation rate of this predatory mite on bulb mite larvae were recorded as 65.0 ± 6.2, during oviposition (Ragusa and Zedan 1988).

Furthermore, Wright and Chambers (1994) showed that *H. miles* feeds on 7.7 ± 1.8 and 0.6 ± 0.2 of first and fourth instar larvae of *Bradysia paupera* Tuomikoski (Diptera:

Sciaridae) per day. They also emphasized that the *H. miles* feed from more earlier larval stages of prey.

Shereef *et al.* (1980) observed that *H. miles* often feeds on mobile stages of prey and have higher efficiency for predation of immobile stages, whereas, *G. aculeifer* unlikely have the ability to attack on mobile prey stages as well (Berndt *et al.* 2004) and it could be a remarkable advantage for a biological control agent, because the mobility of prey is different during different life stages and even short-term situations such as rest or arrestment on food sources.

Brødsgaard *et al*. (1996) investigated the prey preferences of *H. miles* among some glasshouse prey types, including sciarid larvae (*L. solarni*), western flower thrips pupae (*Frankliniella occidentalis* Pergande), eggs and nymphs of Collembola (*Isotomurus* sp.), eggs and mobile stages of mould mites (*T. putrescentiae*), leaf miner pupae (*Liriomyza bryoniae* Kaltenbach) (Diptera: Agromyzidae) and gall-midge pupae (*Aphidoletes aphidimyza* Rondani) (Diptera: Cecidomyiidae) in choice tests. Their results showed that *H. miles* was able to complete its growth and development, and reproduce on all prey species offered, but the most rapid population increase was observed on *L. solani* (r = 0.0747 day^{-1}) and *T. putrescentiae* (r = 0.0543 day^{-1}) (Enkegaard *et al*. 1997). When the leaf miner pupae and gall-midge pupae were provided for this predatory mite, according to the turbidity of hemolymph, cuticle become dark brown that shows the penetration of chelicera of this predatory mite into the pupae. They suggested that *H. miles* prefers sciarid fly of *L. solani* than other preys offered, however, thrips pupae and collembolan nymphs easily were consumed rather than pupal stages of leaf miner and gall midges (Brødsgaard *et al.* 1996).

During this study, it was observed that sciarid larvae greatly tend to accumulate on the food sources after being found, and their body usually covered by straw and debris in the environment that it is likely to retain moisture and helps camouflage. Therefore, one of the important factors effective in choosing the earlier stages of prey is the fewer deterrence response of predator attack by earlier stages that may lead to abandon prey and leave the area. This might be due to the quick and rotational movements of sciarid larvae when predatory mite approaching and contacting them.

It has been recommended that in case of preventive use of ENTOMITE-A product (*G. aculeifer*) against sciarid flies, mite application rates should be 100/m², for light and heavy infection 200/m² and 500/m² respectively (Koppert Biological System 2016). Additionally, Biological Services Company as a commercial insectary recommends customers on the correct application of the predatory mite, that best results are obtained when *G. aculeifer* used precautionary i.e. before the appearance of pest or when the pest population is low (Biological Services 2016).

Lesna *et al.* (2000) tested the efficiency of soil-dwelling predatory mite, *G. aculeifer* to control mites attacking lily bulbs. They observed within the first week after predator release, there was a sharp decline in *R. robini* population up to 10–40% compared with control. Greenhouse experiments showed that *G. aculeifer*, suppressed bulb mite populations when released in a ratio of 1: 2–5 predator: prey.

In another research, five different rates of release were evaluated on *Cyclamen* spp. and *Poinsettia* spp. crops for the control of sciarids (*Bradysia* spp.). Rates of 55 *H. miles* per pot and satisfactorily suppressed sciarid flies population without any outbreak in next generation (Chambers *et al*. 1993).

Collectively, insight in the predator preference would improve our knowledge in timely releasing biocontrol agents against sciarid flies. Rather, the economic damage of herbivores would be inevitable (Shirvani-Farsani *et al*. 2013). According to these results, it has been suggested that releasing of *G. aculeifer* against early instar of *L. auripila* and as a preventive treatment would be reduced *L. auripila* population substantially.

Our research is a first step toward the use of *G. aculeifer* against sciarid fly in Iranian mushroom production. We recommend more comprehensive researches in relation with the ecology, behavior, and release of this efficient predator with sciarids and other potential prey species.

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تعیین الگوی ترجیح غذایی *Gaeolaelaps aculeifer* (Acari: Laelapidae) با تغذیه از پشهٔ قارچ، *Lycoriella auripila*، گامی به سوی رهاسازی کارآمد

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چکیده

پشهٔ قارچ *Lycoriella auripila* Winnertz (Diptera: Sciaridae) یکی از آفات مهم قارچ خوراکی *Agaricus bisporus* (Lange) Imbach است، که به‌واسطهٔ ایجاد کانال در پایهٔ قارچ خوراکی توسط لاروها و نقش بالغ‌ها در انتقال عوامل بیمارگر، در صورت عدم کنترل می‌تواند منجر به کاهش عملکرد زیادی شود. کنهٔ *Gaeolaelaps aculeifer* Canestrini گونه‌ای چندین‌خوار است که در کاهش جمعیت آفات خاکزی از جمله کنه‌ها، نماتدها و قارچ‌ها در اکوسیستم خاک نقش مهمی دارد و امروزه به عنوان عامل کنترل بیولوژیک و شکارگری کارآمد در کنترل تخم و لارو پشه‌های قارچ‌خوار به‌صورت تجاری استفاده می‌شود. با توجه به جایگاه ترجیح غذایی دشمنان طبیعی به عنوان یکی از عوامل موثر در انتخاب مرحلهٔ زندگی طعمه برای رهاسازی و زمان رهاسازی، ترجیح غذایی و نرخ شکارگری این شکارگر در اتاقک رشد در دمای ۱±۲۳ درجه سلسیوس، رطوبت ۵±۶۰ درصد و شرایط ۲۴ ساعت تاریکی بررسی شد. برای بررسی ترجیح از شاخص بتای منلی استفاده شد. نتایج به دست آمده نشان دادکه مرحلهٔ زیستی آفت هدف روی ترجیح غذایی موثر است، به‌طوری‌که میزان تغذیه کنه‌های مادهٔ هفت روزه *G. aculeifer* از لارو سنین دوم و سوم *L. auripila*، به‌ترتیب ۰/۳۳±۵/۰۶۶ و ۰/۲۱±۲/۳۳ و مقدار شاخص منلی روی همین مراحل به‌ترتیب ۰/۰۳۷۷±۰/۶۹۷ و ۰/۰۳۷۷±۰/۳۰۳ به دست آمد. این اختلاف در شاخص ترجیح را می‌توان به اندازهٔ متفاوت لاروهای سنین دوم (۰/۰۸ ±۱/۳۶ میلی‌متر) و سوم (۰/۱۰ ±۲/۵۲ میلی‌متر) نسبت داد. رهاسازی به موقع کنه شکارگر در کارگاه تولید قارچ نتایج بسیار قابل توجهی داشت. هم‌چنین، نتایج به‌دست آمده نشان داد که رهاسازی به‌موقع کنه شکارگر، جمعیت پشه‌های سیارید را کاهش می‌دهد. بنابراین به‌نظر می‌رسد رهاسازی کنه شکارگر

G. aculeifer پیش از فرا رسیدن مرحلهٔ سوم لاروی *L. auripila* ، نتیجهٔ مطلوب‌تری علیه پشه‌های سیارید در تولید قارچ خوراکی خواهد داشت.

واژگان کلیدی: *Agaricus bisporus* ؛ کنترل بیولوژیک؛ شاخص منلی؛ نرخ شکارگری؛ پشه‌های سیارید.

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