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# Incidence and Occurrence of Insect Pest Associated with White Button Mushroom *Agaricus bisporus* (Lange) Imbach in Himachal Pradesh, India

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Abstract: Mushroom flies pose a significant threat to button mushroom cultivation, with sciarid and phorid flies being the most prevalent. This study aimed to assess mushroom fly occurrence in Himachal Pradesh. Surveys were conducted across mushroom farms in Kangra, Kullu, Solan, and Mandi districts, focusing on incidence, damage symptoms, and species presence, particularly *Megaselia scalaris*, *Sciara*, *Sphaerocera*, and *Leptocera*. Sciarid flies were notably abundant. Infestation levels of *A. bisporus* ranged from 9.00 to 38.63 percent across districts. Solan exhibited the lowest infestation (8.25%) in May 2023, while Mandi recorded the highest (38.63%) in March 2022. Infestation primarily targeted compost and casing materials, leading to substantial yield losses and inferior mushroom quality. A prominent sign of damage was brown patches on sporophores, visible on fruiting bodies. This research sheds light on the severity of mushroom fly infestation, crucial for effective pest management in mushroom farming

**Keyword:** *agaricus bisporus* • mushroom flies • incidence • morphometry • image analyzer

## Introduction

Despite the country's diverse agro-climate conditions and a large amount of agricultural and industrial waste, India's mushroom industry is growing slowly compared to other mushroom-growing countries. Mushrooms are recognised as an alternate source of high-quality protein (Kalač, 2013), with the ability to produce the protein per unit area from agricultural waste (Kakraliya, 2022), and as a rich source of vitamins (Heleno et al., 2010; Mattila et al., 2001). *Agaricus bisporus* is the most common cultivated edible mushroom, consumed for its delicacy, flavour, and agreeable consistency, in addition to having nutritive and therapeutic benefits due to simple, low-cost cultivation technology (Deepalakshmi & Mirunalini, 2008).

Mushroom farming encounters considerable obstacles due to a variety of pests and diseases, with dipteran species such as phorid and sciarid flies being particularly notable among them. Both adult and immature flies from the Diptera order are identified as nuisances in mushroom cultivation, causing damage to developing hyphae through various means. This includes immature flies feeding on fruiting bodies (Erler & Polat, 2008; Hosaka & Uno, 2012), vectoring mites (Birkemoe et al., 2018), or directly damaging hyphal development and pinheads (Gratwick, 1992, Firake et al., 2018). These actions ultimately result in production and yield losses. Moreover, they can act as vectors for pathogenic fungi and mites (Shamshad et al., 2008), exacerbating the impact on mushroom production.

Historically, sciarids were considered the most significant pests; however, phorids, particularly *Megaselia halterata*, have gained attention due to their increasing prevalence as major pests in countries like India, the UK, and the USA, where they have caused yield losses ranging from 10 to 40 percent (Navarro et al., 2021). During the growth season, adult flies can also support pest populations by ovipositing. The damage threshold for commercial mushroom production is 108 females of *Lycoriella mali* per square meter. Four larvae per square meter during a spawning run can significantly reduce output, according to earlier (White, 1990; Wyatt, 1978) Due to flies' intrinsic ability to reproduce, up to 3.5 larvae per square meter have been recorded. It has also been proven that a mean of one sciarid larva in 125 g of casing results in a 0.5% reduction in overall yield. An economic threshold for this pest is represented by the cost of the advised sciarid control measures, which is likewise 0.5% of the value of the crop (Singh & Sharma, 2016).

Mushrooms that are infested frequently lose their color, become brown, grow leathery, and become unusable. Due to the presence of maggots and pupae in the produce, post production damages significantly diminish marketability in addition to reducing the quantity and quality of mushrooms. A proficiency in species identification and their developmental stages is necessary for the creation of effective pest population management strategies. Morphometric approaches are frequently utilized in studies on a variety of insect groups to achieve this goal.

**Materials and methods:**

Collection of mushroom flies: Samples of immature stages of insects were collected from composting ingredients as well as cropping beds in fresh polythene bags, sealed properly with rubber bands to avoid moisture loss, and labelled for the locality, cropping time, and date of collection. The life stages of pests found during the survey were preserved in 70 percent alcohol. Samples brought to the laboratory were processed to study the incidence of insects. Precaution were taken to maintain the optimum moisture level until the analysis was complete. Adult insects hovering over the cropping bags and inside the mushroom farms were collected by different methods. The labeling of cages was also done for location and date of collection. Water was sprinkled daily in the cages to maintain optimum moisture levels. Adult flies hovering over the bags were collected and preserved separately in plastic vials of 5 ml capacity containing 70 percent alcohol for identification purposes. Visible symptoms of fly infestation were also recorded during this survey study.

**Identification of Species**

All the species collected were photographed, and mounts of different body parts, viz., antennae, legs, wings, and claspers, were also made. Morphometric analysis was done by image-analyzing software. Identification was done by using taxonomic keys, and for further confirmation, preserved adult flies were sent to the Zoological Survey of India, Kolkata.

**Identification of symptoms in damaged mushroom**

The fruiting bodies of the white button mushroom showed the most obvious signs of injury. As a result, fruiting bodies were removed from cropping bags infested with sciarid flies from several mushroom units investigated to look for signs of damage.

**Morphometric analysis of mushroom flies**

Using a stereo microscope and image-analyzing software, the length and width of different parts of mushroom flies, viz., legs, wings, antennae, etc., were analyzed three time and the same was photographed.

## Results

The purpose of the current study was to determine occurrence of *Agaricus bisporus* associated with mushroom flies. Twenty mushroom farms in various regions of Himachal Pradesh were surveyed to determine the frequency of flies in *Agaricus bisporus* cultivation units. In all of the mushroom units investigated, it was discovered that numerous dipteran flies of several families, including Sciaridae, Phoridae, and Sphaeroceridae, were present.

**Incidence of major mushroom flies on *Agaricus bisporus*:** A comprehensive study was done on the occurrence of mushroom flies in *Agaricus bisporus* growing different regions of Himachal Pradesh. Samples of flies were gathered from Kullu, Mandi, Solan, and Kangra districts along with geographical factors like longitude and latitude. In the previous year, sixteen farms were surveyed using a putter and taking samples of the compost at each stage of the agricultural cycle. These samples, which included discarded compost, spawning compost, casing material, and fruiting bodies, were taken from small to large mushroom farms. Different genera of fly were observed in different mushroom farms including *Sciara*, *Megaselia scalaris*, *Sphaerocera*, and *Leptocera*. It was observed that the major mushroom fly infestation on *A. bisporus* ranged from 9.00 to 38.63 percent. Out of the four districts examined, Solan had the lowest percent infestation (8.25%) during May 2023, while Mandi district recorded the highest percent infection (38.63%) during March 2022 followed by Kangra (38.23%), and Kullu (34.22%). Kakraliya (2022), who surveyed 59 mushroom farms in Jammu, and reported that 6.77 percent of farms were infested with beetles, while 17.79 percent of farms were infested with phorid flies, and 11.01% farms showed infestations of sciarid flies.

**Table 1.** Incidence of mushroom flies in *A. bisporus* in different districts of Himachal Pradesh

| Location | Survey Units | Coordinates | Percentage incidence | Mean fly incidence |
|---|---|---|---|---|
| Kullu | Mali Pathar | 32°3'10.13" N, 77°7'34.03" E | 38.57 | 34.22 |
| | Neoli | 31°58'42.28" N, 77°7'48.708" E | 36.75 | |
| | Bandrol | 32°1'13.44" N, 77°7'33.92"E | 19.54 | |
| | Bajora | 31°50'24.73" N, 77°9'41.273" E | 42.00 | |
| Mandi | Patyani | 31°33'29.52" N, 76°57'53.77" E | 37.83 | 38.63 |
| | Chakhara | 31°33'7.13" N, 76°55'0.31"E | 43.06 | |
| | Salwahad | 31°34'9.589" N, 76°57'8.01" E | 35.00 | |
| Kangra | Kandwal | 32°17'18.336" N, 75°47'5.179" E | 10.00 | 32.83 |
| | Nakodar Fatehpur | 32°12'15.987" N, 75°55'49.592" E | 37.5 | |
| | Bhol Jawali | 32°8'44.462" N, 75°59"7.399" E | 53.84 | |
| | Ban Khandi | 31°57'33.205" N, 76°12'10.071" E | 30.00 | |
| Solan | Shattal | 30°56'23.1183"N, 77°2'3.6639" E | 10.00 | 8.25 |
| | Nauni Greti | 30°56'38.8608"N,77°2'22.42212" E | 9.00 | |
| | Lakharnji | 30°54'1.19304"N, 77°10'1.38432" E | 4.00 | |
| | Mashiwer | 30°54'22.5403"N, 77°9'38.54844" E | 10.00 | |

## Damage Symptoms

The fruiting bodies showed clear signs of infestation, with larvae boring into the stipe from compost and creating tunnels (Fig.1A, 1B) to reach the fruiting bodies, while adult flies generated brown patches on sporophores that rendered them unfit for sale. The majority of the larvae were discovered on the compost and casing material; however, when the fruiting bodies were broken open, a significant number of larvae were discovered inside (Fig.1C). Fly-infested bags did not yield high-quality mushrooms and caused significant yield loss. As a result, the yield loss of the mushroom crop was deemed to be equivalent to the pest incidence.

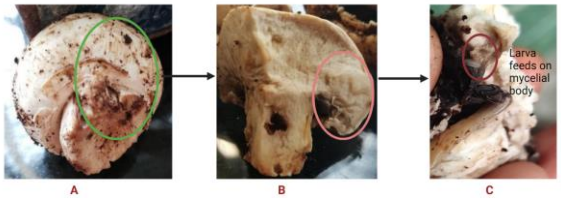


**Fig. 1.** Symptoms of damage caused by mushroom flies associated with *Agaricus bisporus*,

(A) represents a tunnel on the stipe towards the pilus, (B) represents wilting of the fruiting body, (C) represents larvae feeding on the fruiting body of *Agaricus bisporus.*`

## Morphometric analysis of mushroom flies

The known white button mushroom feeders were flies belonging to the families Sphaeroceridae, Sciaridae, and Phoridae. These

were also the families of flies reported by other researchers as being associated with mushroom cultivation (Coles et al., 2021; Erler & Polat, 2015; Fiegalan, 2020; Singh & Sharma, 2016). The flies found associated with *Agaricus bisporus* cultivation represented both suborders Nematocera and Brachycera, signifying the breadth of dipteran diversity. Of the suborder Nematocera, only one family was represented i.e., Sciaridae. The suborder Brachycera is segregated into two series, Aschiza and Schizophora. Phoridae was the family represented in the series Aschiza, while Schizophora included the Sphaeroceridae family.

Of the four species found, one was identified at species level, and the other was identified as genus level. The three species identified at the level of genera were *Sphaerocera sp.*, *Sciara sp.*, and *Leptocera sp.* The one species identified at the level of genera was *Megaselia scalaris.* The following details the taxonomic treatments for these four species.

**Table 2.** List of adult flies from *Agaricus bisporus* mushroom beds, showing the classification, taxon, and morphometric parameters of different flies observed in Himachal Pradesh.

| Suborder | Division | Series | Family | Genera | Morphometric analy mm) |
|---|---|---|---|---|---|
| **Nematocera** | - | - | Sciaridae | *Sciaria* sp. | Egg Length = 0.25, Egg 0.13, Larvae = 1.87 ,WL WW = 0.54 Foreleg Antennae = 0.61 |
| **Brachycera** | Cyclorrhap ha | Schizopho ra | Sphaerocerida e | *Leptocera* sp. | Egg Length = 0.18, Egg 0.09, Larvae = 1.43 ,WL WW = 0.21, Foreleg Antennae = 0.34 |
| **Brachycera** | Cyclorrhap ha | Schizopho ra | Sphaerocerida e | *Sphaerocer a* sp. | Egg Length = 0.20, Eg = 0.11, Larvae = 1.52, 1.09, WW = 0.52, Fo 1.35, Antennae = 0.82 |
| **Brachycera** | - | Aschiza | Phoridae | *Megaselia scalaris* | Egg Length = 0.22, Egg 0.11, Larvae = 1.72, WL WW= 0.56, Foreleg Antennae = 0.41 |

## Family Sciaridae

*Sciaria* sp. commonly known as dark-winged fungus gnats. This family was widespread, with members adapted to a wide variety of climates. Adults of this family had small, dark wings with characteristic venation; there was no cross vein except for a short rs at the wing base, and the anterior veins were a short $R_1$ and a long $R_5$ (Fig 2A). The antennae were thread-like and filiform in shape (Fig. 2C). Coxa, trochanter, and femur are yellow brown, while tibia are slightly darker (Figure 2B). The tibia had more hair than the coxa, the trochanter and the femur. The wing length (WW), wing width (WW), front leg and antenna were measured at 1.04 mm, 0.54 mm, 1.06 mm and 0.61 mm, respectively. The gonostyles are enlarged and have short, hair-like subapical spines; the genitalia (Fig. 2D) have sparse, strong, and long hairs on the inner edge of the gonocoxites.

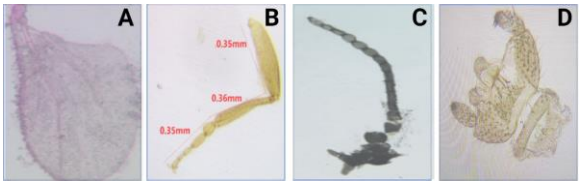


**Fig. 2**. Adult of Sciaria species (A-D) (A) Wing (B) Leg (C) Antennae (D) Genitalia

## Family Sphaeroceridae

*Leptocera* sp.: Adults of these flies were brownish-black, small to very minute flies, with wings usually present; Sc was either indistinct distally, or not ending in the costa independently of R1, or joined to R1 by sclerotization of the intervening region (Fig.3A). The coxae of each pair were not separated, the proboscis developed, the forelegs were not raptorial, and the tarsal claws normal. The wing length (WL), wing width (WW), foreleg (Fig.3B), and antennae (Fig 3C) were measured as 0.40mm, 0.21mm, 0.61mm, and 0.34mm, respectively.


**Fig. 3**. Adult of Leptocera species (A-D) (A) Wing (B) Leg (C) Antennae (D) Genitalia

***Sphaerocera* sp.**

Adults were generally black in color, wing with R4+5 and M gradually converging towards the apex and a normal anterior crossveins, CuA1 reaching the wing margin, an antenna yellowish brown, and a hind tibia with a well-developed venter-apical spur . The wing length (WL), wing width (WW) (Fig.4A), foreleg (Fig. 4B), and antennae (Fig.4C) were measured as 1.09mm, 0.52mm, 1.35mm, and 0.82mm, respectively.

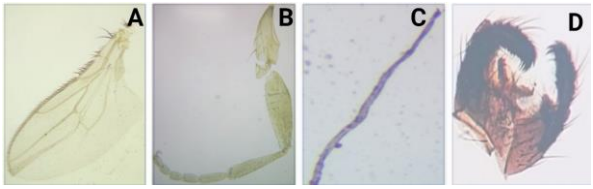


**Fig. 4**. Adult of *Sphaerocera* species (A-D) (A) Wing (B) Leg (C) Antennae (D) Genitalia

Family Phoridae

*Megaselia scalaris* Commonly called hump-backed fly, small or minute, wing venation was reduced, well-developed radial (R) veins end in the costa about halfway along the wing, crossveins are totally absent, and R4+5 may furcate at the end (Fig.5A). The wing length (WL), wing width (WW), foreleg (Fig 5B), and antennae (Fig.5C) were measured as 1.27mm, 0.56mm, 1.49mm, and 0.41mm, respectively.

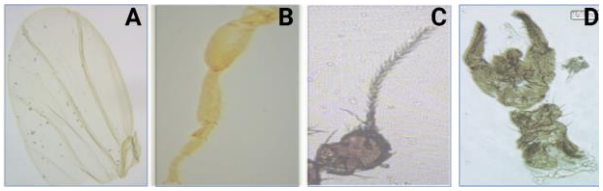


**Fig. 5**. Adult of *Megaselia scalaris* (A-D) (A) Wing (B) Leg (C) Antennae (D) Genitalia

## Discussion

Mushroom crops are susceptible to various insect pests, including mushroom flies. These pests can significantly impact on mushroom yields and quality. The incidence of insect pests in mushroom cultivation varies with environmental conditions, mushroom species, and geographical location. Monitoring and early detection are essential to assess the incidence of these pests. This can be done through regular inspections of mushroom beds and traps. Accurate data on pest incidence and pest species identification helps to growers to implement targeted control measures, ensuring the success of their mushroom crops.

The results of our study suggest that the presence of maggots on the sporophores of white button mushrooms can lead to significant economic losses in terms of reduced yield. It is crucial for mushroom growers to implement effective pest management strategies to control the infestation of *L. ingenua* and *B. ocellaris*, as their larvae can cause extensive damage to the crop. The presented results showed that the average infestation on *Agaricus bisporus* ranged from 9.00 to 38.63 percent. Out of the four district, Solan had the lowest percent infestation i.e. 8.25% during May 2023, while Mandi district recorded highest percent infestation i.e. 38.63% during March 2023 followed by Kangra (38.23%) and Kullu (34.22%). Fernandes (2016) reported 17 species belonging to eight genera in Colombia. Gnaneswaran and Wijayagunasekara (1999) reported comparable damage caused by larvae of the sciarid fly. These larvae infiltrated the stalk of oyster mushrooms, ultimately affecting the young fruiting bodies, causing them to wilt. Similar results were also reported by Sharma (2010) regarding the adverse effects of *Sciara* species who observed the emergence of brown patches on the sporophores of white button mushrooms. Along with development of tunnels on the stipe, leading towards the pileus of these emerging sporophores. A significant number of maggots were released when dissected. Shamshad (2010) observed substantial yield losses due to *L. ingenua* and *B. ocellaris*, both belonging to the Sciaridae family of diptera and also found a direct correlation between the damage caused by these fly larvae and the resulting yield losses.

Mushroom fly was identified at the species level and the other at the genus level out of the four that were discovered. *Sphaerocera* sp., *Sciara* sp., and *Leptocera* sp. were the three flies that were identified down to the genus level. *Megaselia scalaris* was the only fly identified at species level. *Megaselia scalaris*, also referred to as the coffin fly, was found to be the species identified at the species level. This species is a member of the Aschiza series and is in the Phoridae family. One of the genus-level identifications was *Sphaerocera* sp., a member of the Schizophora series' Sphaeroceridae family. The two further genus-level identifications were *Leptocera* sp. and *Sciara* sp., both of which are members of the Phoridae family and belong to the Aschiza series. In a two-year survey study carried out in the Antalya-Korkuteli district, three sciarid species, namely *Lycoriella auripila, L. ingénue,* which was the first record for the Turkish fauna, and *L. solani*, were detected in mushroom-growing farms in this district (Erler & Polat, 2015). Sharma et al. (2019) conducted a faunistic survey of pests associated with *A. bisporus* in four districts of Himachal Pradesh and reported that the highest population of flies was in Kangra district and the lowest was in Kullu district. During another survey on mushroom flies, *Megaselia scalaris* was observed to be the most common species on the mushroom-growing farms (Lewandowski et al., 2004).

## Conclusion

The study, conducted across mushroom farms, revealed the presence of various dipteran flies like sciarid, phorid, leptocera, and sphaerocera flies, recognized as significant pests infesting mushroom crops. Further research, particularly on large-scale *Agaricus bisporus* cultivation, could offer precise insights into fly population dynamics and yield loss assessment. Understanding these dynamics is pivotal for effective pest management strategies. By delving into extensive studies on *Agaricus bisporus* cultivation, researchers can gain nuanced understandings of fly behavior and population trends. This knowledge can facilitate targeted control measures, alleviating severe infestations and enhancing overall productivity and profitability of mushroom farms.

Author's contribution: Kumari Ruchika: Writing- original draft, Review and Editing, Prof. Sunil Kumar: Conceptualization, Methodology, Editing, Supervision, Dr. Sachin Upamanyu: Editing, Supervision, Kanika Choudhary: Visualization, Formal analysis, Jigmet Yangchan: Writing and editing, Poonam Kumari: Data curation, analysis, Alka Rana: Editing, Data curation.

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