INCIDENCE, BIOLOGY AND BIO-INTENSIVE MANAGEMENT OF DIPTERAN FLIES IN OYSTER MUSHROOM (*Pleurotus* spp.)

Thesis

by

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(NH-2018-04-M)

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DR. YASHWANT SINGH PARMAR UNIVERSITY
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CERTIFICATE - I

This is to certify that the thesis entitled "Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)**",** submitted in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY** in the discipline of **PLANT PROTECTION** of Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) – 173230 is a bonafide research work carried out by **Ms. DEVIKA SHARMA (NH-2018-04-M)** daughter of Shri Neeraj Sharma under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation has been fully acknowledged.

Place: Neri, Hamirpur
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CERTIFICATE - II

This is to certify that thesis entitled **"Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)"** submitted by **Ms. Devika Sharma (NH-2018-04-M)** daughter of Shri Neeraj Sharma to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry (Nauni), Solan (HP)-173230 India, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY** in the discipline of **PLANT PROTECTION** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an internal examiner.

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This is to certify that all the mistakes and errors pointed out by the external examiner have been incorporated in the thesis entitled **"Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)"** submitted by **Ms. Devika Sharma (NH-2018-04-M)** daughter of Sh. Neeraj Sharma to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni (Solan) -173 230 H.P. in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY.**

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I own entire responsibility for all the errors and omissions.

Place: Neri, Hamirpur
Dated: **(Devika Sharma)**

CONTENTS

LIST OF ABBREVIATIONS USED

H	:	Hour
@	:	At the rate of
%	:	Per cent
/	:	Or
a. i.	:	Active ingredient
CD	:	Critical difference
ANOVA	:	Analysis of variance
etc.	:	Etcetera
Ml	:	Milli liter
G	:	Gram
Mg	:	Milli gram
Cm	:	Centimeter
Mm	:	Milli meter
Ha	:	Hectare
CRD	:	Completely Randomized Design
L	:	Liter
LC_{50}	:	Lethal Concentration 50
LC_{90}	:	Lethal Concentration 90
spp.	:	Species
SE	:	Standard Error
viz.	:	Namely
i.e.	:	That is
Ppm	:	Parts per million
conc.	:	Concentration
°C	:	Degree Celsius
MT	:	Metric ton
RH	:	Relative humidity

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Chapter -1

INTRODUCTION

Mushrooms are the macro fungi with distinct fruiting body and are often found as saprophytes on soil, open fields, farm lands, wood and roadsides. They are a good source of protein, minerals and vitamins and are known to have a broad range of uses both as food and medicine (Josphine, 2015). The area under mushroom cultivation in India is 198,000 ha with an annual production of 487,000 MT (Anonymous, 2017). Mushroom cultivation can make a valuable contribution to sustainable livelihoods because they require minimal physical and financial resources. In India, only 3 species, namely, *Agaricus bisporus*, *Pleurotus sajor-caju* and *Volveriella* are preferred for commercial cultivation. Out of the total mushrooms produced in India, the share of oyster mushroom is 16 per cent (Sharma *et al*., 2017). Oyster mushroom ranks second among the cultivated mushrooms in the world (Sharma and Singh, 2018).

Oyster mushroom (*Pleurotus* spp.) belonging to Class Basidiomycetes and Family Agaricaceae, popularly known as 'Dhingri' in India, grows naturally in the temperate and tropical forests on dead and decaying wooden logs or sometimes on dying trunks of deciduous or coniferous woods (Randive, 2012). They were first cultivated on tree logs, and are now commonly grown on sawdust, wheat or rice straw, which has shortened the fruiting period to about two months. The Latin *Pleurotus* refers to the sideways growth of the stem with respect to the cap. The fruit bodies of this mushroom are distinctly shell or spatula shaped with different shades of white, cream, grey, yellow, pink or light brown depending upon the species.

Cultivation of oyster mushroom requires a moderate temperature ranging from 20-30°C, with high relative humidity 75-85 per cent during fruiting (Baiswar *et al*., 2016). Oyster mushrooms are known to have therapeutic ingredients such as dietary fibres (chitins and chitosans) and phenolic compound (Neyrinck *et al*., 2009). It is a rich source of vitamin C & B complex and contains mineral salts required for the human body. Apart from that, it has a high protein content of 1.6 to 2.5 per cent. The niacin content is about ten times higher than any other vegetables. The folic acid present in oyster mushrooms helps to cure anemia. It is suitable for people with hyper-tension, obesity and diabetes (Randive, 2012).

Mushrooms are attacked by a number of pests from spawning to harvest. Since mushrooms are grown mostly in an enclosed environment, the risk of insect pests and disease spreading rapidly within the crop are high as they find very suitable and protected environment for their unhindered multiplication. Various insects, mites and nematode pests feed on oyster mushroom in India at different growth stages and cause extensive losses in the yield and even sometimes cause total crop failure (Deepthi *et al.*, 2004). Among insect pests; sciarid fly, phorid fly, cecid fly, nematodes, springtails, mites, beetles, moths etc. cause damage to the crop (Singh and Sharma, 2016). These insects are potential crop threats which are attracted by the odour of the substrate. Among all, *Bradysia* spp. and *Triplax* spp. were found to be the most destructive pests of oyster mushroom (Nongkynrih *et al.*, 2017). Flies, especially in their immature stage (larvae), perforate the stipe and pileus of mushrooms, opening inside galleries and consequently causing its overall depreciation. Larval feeding of second and the subsequent generations can be very destructive. Dipterous larvae can derail the product for consumption, leading to losses of around 20 per cent. Beetles were also observed damaging oyster mushroom. The adults are attracted to the smell of decayed mushrooms (Singh and Sharma, 2016). Springtails are generally associated with the decomposition of organic matter. However they can become a nuisance in *Pleurotus* mushroom production as it feeds on gills of the mushroom (Mignucci *et al.*, 2000). Several groups of mites have been associated with mushroom cultivation and cause destruction of mycelium along with surface discoloration (Rinker, 2017).

Bhattacharyya *et al*., 1993 reported that the yield of mushrooms was reduced by 49 per cent due to the pest attack. Pest incidence was found upto 100 per cent during the rainy season (May, June, July and August) (Nongkynrih *et al*., 2017). Infestation by order Collembola (springtail) on *P. ostreatus* was found to be higher in incidence with per cent composition of 50.0 (%), followed by order Diptera (fruit flies) and order Araneae (spider) with 33.3 (%) and 16.67 (%) composition respectively (Oyebamiji *et al*., 2018).

Such huge losses incurred by these insect pests necessitates the use of ecofriendly management strategies against them. The use of pesticides for killing insect pests in mushroom crops is not advisable due to residual hazards as mushroom is a short duration crop. Hence, there is a need to exploit the use of some plant products, which have insecticidal properties and are readily biodegradable, suitable for use by farmers and yet are capable of protecting crops from

attack by a wide range of insect pests. The benefical effects of the use of predators, insect parasitoids, fungus, bacteria and entomopathogenic nematodes have been observed for the control of mushroom flies. The use of certain type of microbial bio-agents have been attributed to decrease the population densities of insect pests of mushroom. Several control methods have been worked out under Indian conditions and use of plant products were more successful in minimizing pest damage to mushroom and maximizing the yield. Plant-derived products (neem, eucalyptus, darek, garlic, anise, etc.) could be recommended to substitute the synthetic chemicals in the commercial production of edible mushrooms. Plant-based products in various formulations have been found to be effective due to their different modes of action against insects and mites; *i.e*. antifeedants, pesticides, growth regulators, repellents, and oviposition deterrents (Gahukar, 2014).

Keeping in view, the above facts, the present investigation entitled, **"Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)"** has been proposed with the following objectives:

i. Incidence and identification of different insect pests in oyster mushroom,

ii. Biology of two major insect pests recorded during the survey studies and

iii. Eco-friendly management of two major insect pest species of oyster mushroom.

Chapter -2

REVIEW OF LITERATURE

A few important references contributive to the related research work that could be of some significance are presented here:

2.1 Incidence of insect pests of oyster mushroom in mushroom growing localities of subtropical areas

Johal and Disney (1994) discovered that in India the cultivated oyster mushroom *Pleurotus sajor-caju* Singer was attacked by the larvae of two species of Phoridae, *Megaselia pleurota* Disney and *M. scalaris* (Loew). The new species identified was described and biological data and damage symptoms were reported for both species. Serious economic damage caused by a bacterium associated with *M. scalaris* was also reported.

Mohan and Disney (1995) reported that the cultivated oyster mushroom *Pleurotus citrinopileatus* Singer, was attacked by larvae of the phorid fly *Megaselia tamilnaduensis* Disney in South India. The larvae fed on the mycelium. When more than 3 per cent of the area of the surface of 2.5 kg cylindrical mushroom beds had been damaged the subsequent yields of sporophores fell from 400-500 g to 200-350 g. The new species has been described and biological data summarized.

Gnaneswaran and Wijayagunasekara (1999) surveyed and identified insect pests of oyster mushroom (*Pleurotus ostreatus*) cultures in central province of Sri Lanka. Nine species of insects were found to be associated with mushroom cultures. Among them only three species namely *Drosophila funebris* F., *Bradysia papuera* Tuomikoski and *Cyllodes bifacies* (Walker) were found to cause some damage. *Cyllodes bifacies* was distributed in about 96 per cent of the farms visited causing serious damage up to 82 per cent. It was identified as the key pest of mushrooms grown in the Central Province.

Navarro *et al*. (2002) studied evolution and seasonal abundance of phorid and sciarid flies in Spanish mushroom crops. The mushroom fly populations of twenty-four mushroom crops in

Spain were studied for eighteen months. The study was carried out by monitoring both adult and immature stages. The relative importance of *Megaselia halterata* and *Lycoriella auripila* in each season, their evolution during the crop cycle and possible access routes to mushroom farms were discussed. The highest number of adult flies was collected in spring and autumn, while in winter inspite of decrease in numbers, they did not completely disappear from the growing crops.

Greenslade and Clift (2004) recorded twenty six arthropod species (nine Diptera, ten Acari, seven Collembola) as mushroom pests in Australia. The species included one new record for Australia, *Bradysia difformis* Frey, 1948 and one new record for Tasmania, *Lycoriella ingenua* Frey, 1948. All the species discovered had cosmopolitan or nearly cosmopolitan distribution.

Cline and Leschen (2005) presented a checklist of the beetles known to occur in and on the oyster mushroom, *Pleurotus ostreatus* Fries. A total of 136 taxa from 30 families were presented by them. Both adult and larval forms were presented in their checklist along with the type of fungal association. A total of 58 beetle taxa were summarized from existing published records, and 78 taxa were newly added.

Joshi (2009) screened six species of *Pleurotus*, namely, *P. sajor-caju*, *P. florida*, *P. ostreatus*, *P. sapidus*, *P. eous* and *P. eryngii* for the incidence of sciarid fly, *Bradysia tritici* and phorid fly, *Megaselia sandhui* during February-March (2008 and 2009). There was moderate infestation by sciarid fly larvae except on *P. eous* which showed high infestation by sciarid fly larvae, while all the species were highly infested by phorid fly.

Jonathan *et al*. (2012) collected insect and fungal pests of some mushrooms from university of Ibadan, Nigeria. Ten mushrooms species were collected within the premises of University of Ibadan and examined for infestation of various insect and fungal pests. Insects belonging to the orders; Coleoptera, Hymenoptera, Diptera, and Collembolla were encountered both at the larval and adult stages of life on the collected mushroom samples. Infestation by the order Coleoptera (adult beetle) on *Pleurotus squar-rosulus* was found to be higher in incidence, with a total number of 17 species which were found at the adult stage of life.

Shin *et al.* (2012) collected seven species of sciarid flies in shiitake mushroom farm in Korea. Among them, *Lycoriella ingenua* Dufour 1839 and *Bradysia difformis* Frey 1948 were

dominant as possible pests of the shiitake mushroom because the larvae were found on both oak bed logs and in the artificial sawdust beds for shiitake cultivation.

Nongkynrih *et al*. (2017) studied pest complex of cultivated oyster mushroom in Northeast India, their feeding losses and role of micro-climate in pest multiplication. Various arthropod pests *viz*., pleasing fungus beetles, sciarid fly (*Bradysia* spp.), mycetophilid fly, fruit fly, rove beetles, noctuid moths, collembolans and mites were found infesting oyster mushroom in different months of the year. Pest incidence was found upto 100 per cent during the rainy season (May, June, July and August). Among all, *Triplax* spp. and *Bradysia* spp. were found to be the most destructive pests of oyster mushroom; which were present throughout the year and in turn were responsible for reduction in the marketable yield.

Bellettini *et al*. (2018) described diseases and pests noxious to *Pleurotus* spp. All the important bacterial, fungal, and viral diseases were described. Pests causing damage to oyster mushroom including flies, beetles, springtails, mites, nematodes, rodents, termites and mollusc have been explained so.

Oyebamiji *et al.* (2018) examined fungal and insect pests of the edible mushroom *Pleurotus ostreatus.* Various insect pests on *P. ostreatus* were collected and analysed. Insects belonging to the orders Collembola, Diptera and Araneae were encountered on the cultivated *P. ostreatus*. These groups of insects were found at adult stage of life. Infestation by order Collembola (springtail) on *P. ostreatus* was found to be higher in incidence with percentage composition of 50.0 (%), followed by order Diptera (fruit flies) and order Araneae (spider) with 33.3(%) and 16.67 (%) composition, respectively. Distinguishing features of each pest and the features of damage done on mushroom were also observed and recorded.

Katumanyane *et al.*, 2020 conducted a survey of eight greenhouses in the Western Cape Province (South Africa) and found all the greenhouses being attacked by *B. impatiens*, which indicated multiple strains of the fly in South Africa. Apart from that two more species of flies, *L. sativae* and *L. ingenua* were also found which were seen for the first time in South Africa**.**

2.2 The biology of insect pests of the mushroom

Hussey and Gurney (1968) described the life history of *Lycoriella auripila* in mushroom houses causing damage to the crop at different stages. They also tested different insecticidal formulations, out of which Malathion 0.01 per cent was found to be the best against the larvae in casing.

Choi *et al*. (2000) conducted in vitro evaluation of the development and life history characteristics of *Coboldia fuscipes* (Diptera: Scatopsidae) causing damage to oyster mushroom. They observed ovipositional period, number of eggs laid, larval period, pupal period and adult longevity etc.

Frouz and Novakova (2001) studied the biology of sciarid fly *L. ingenua* in laboratory in Petri plates and reared them on pure culture of the fungi *Absidia cylindrospora* or *A. glauca* on malt extract agar media. Fresh culture was provided for egg laying and larval feeding. The egg development stage lasted for 3.0-5.0 days in case of *L. ingenua* and 1st and 2nd instar took 2.0-3.0 days and 3.0-5.0 days, respectively. The pupal period lasted for 1.0-2.0 days and adults died soon after reproducing and development period of adults was 5.0-7.0 days. Total lifecycle was completed in 18 days at 25°C. On an average, 53 eggs were laid by a single female.

Nigro *et al.* (2007) examined the sex ratio of *Sciara ocellaris* at different temperatures and concluded that it was temperature dependent. At 18-20°C temperature, sex ratio was symmetrical, with equal number of male and female flies emerged. But when temperature was increased to 24-28°C, the number of female flies also increased and number of the male flies was reduced to 30-37 per cent.

Kuhne and Heller (2010) used a simple mass rearing technique by using coconut coir or wood fiber as substrate and added natural oat flakes. The mycelium subsequently growing on oat flakes provided an ideal food source for fungus gnats. Their life history development was recorded and different methods were used to control them such as nematodes (*Steinernema feltiae),* predatory mites (*Hypoaspis miles* or *H. acaulifer*) or bacteria (*Bacillus thuringiensis* var. *israelensis*). Among all the biological pesticides, Neem extracts (*Azadirachta indica*) were the most effective followed by Quassia extracts (*Quassia amara*).

Lewandowski *et al.* (2012) provided insights into morphology, biology and development of *Megaselia halterata* (Wood). Traits such as body length, body weight and width of pseudocephalon, across the subsequent development stages as well as time were discussed. The development time of a generation, from egg to adult, lasted 16-19 days at 24 °C; for larval stage this time lasted 12-14 days. Mean weight of particular stages ranged from 0.003 mg for eggs up to 0.492 mg for pupae, while mean length from 0.35 mm for eggs to 2.73 mm for 3rd instar larvae. During larval development, mean body weight increased about 48 times and mean body length three times. Measurements of pseudocephalon of larvae showed that between the successive instars it increased approximately 1.4 times.

Katumanyane *et al*. (2018) described *Bradysia coprophila* Lintner and *Bradysia impatiens* Johannsen as most important pests of mushrooms. Their life cycle has been explained and their management has been achieved through the use of chemical insecticides and to a considerable extent through the use of bio control agents such as entomopathogenic nematodes (EPNs).

Ziling *et al.* (2018) recorded a new species *Chonocephalus depressus* Meijere, (Diptera: Phoridae), whose larvae were attacking oyster mushroom in China. *Chonocephalus depressus* was redescribed and illustrated, and its biological characteristics were described.

2.3 The efficacy of botanicals, entomopathogenic nematodes and microbial bio-agents against insect pests of mushroom

Cantwell and Cantelo (1984) evaluated *Bacillus thuringiensis* var. *israelensis* against a sciarid fly *Lycoriella mali* in mushroom compost and observed over 90% control. The LC_{50} was one part *B. thuringiensis* var. *israelensis* to 696 parts water (wt/ wt); the LC_{95} was one part *B. thuringiensis* var. *israelensis* to 64 parts water. With a 1: 60 dilution, the mortality exceeded 99.5%.

Osborne *et al*. (1985) tested the effects of *Bacillus thuringensis* var. *israelensis* (*Bti*) against the fungal gnat, *Bradysia coprophila*, in the laboratory and the greenhouse. When insects were exposed to *Bti* (50.9 IU/ cm^2) from egg to pupa, only 8 per cent of the insects survived compared to 84 per cent survival of insects treated only with water. Sub lethal doses of *Bti* retard development

of late instars. B. *thuringiensis* var. *kurstaki* had minimal activity on this insect in comparison to *Bti.*

Mignucci *et al.* (2000) in Puerto Rico found that the insects causing deterioration of *Pleurotus* basidiocarps include springtails, *Lepidocyrtus ramose,* and to a lesser degree, Sciarid and Phorid flies. They designed an integrated sustainable management program without pesticides based on prevention. The program included: optimum substrate pasteurization and sanitary conditions; insect control by barriers, traps and building design; removal of diseased or contaminated units; sustaining optimum environmental requirements and reduction of cropping cycles.

Keil and White (2004) described the use of insect parasitoids (*Synaldis concolor, Muscidifurax raptor, Muscidifurax zaraptor),* entomopathogenic nematodes, predatory mite (*Parasitus bituberosus*), fungal pathogens (*Pandora gloeospora*), bacteria (*Bacillus thuringiensis* var *israelens*) and other considerations of pest management such as physical exclusion of insects and monitoring fly populations for the management of mushroom pests.

Tapondjou *et al.* (2005) extracted essential oils from *Eucalyptus saligna* and *Cupressus sempervirens* leaves and analysed along with cymol, one of the main constituents for their repellent and toxic effects on *Sitophilus zeamais* and *Tribolium confusum.* Contact toxicity assayed by impregnation on filter paper discs or coating onto maize grains showed that these chemicals caused significant mortality of the test insects. *Eucalyptus* oil was more toxic than *Cupressus* oil to both insect species on filter paper discs, and was more toxic to *S. zeamais* on maize. These results suggested that the essential oils from *E. saligna* and *C. sempervirens* may be used in grain storage against insect pests.

Park *et al.* (2006) derived plant essential oils from 40 plant species were tested for their insecticidal activities against larvae of *Lycoriella ingenue* (Dufour) using a fumigation bioassay. Good insecticidal activity against larvae of *L. ingenua* was achieved with essential oils of *Chenopodium ambrosioides* L., *Eucalyptus globulus* Labill, *Eucalyptus smithii* RT Baker, horseradish, anise and garlic at 10 and 5μLL^{-1} air. Horseradish, anise and garlic oils showed the most potent insecticidal activities among the plant essential oils. At 1.25μLL^{-1}, horseradish, anise

and garlic oils caused 100, 93.3 and 13.3% mortality, but at 0.625µLL^{-1} air this decreased to 3.3, 0 and 0% respectively.

Batish *et al.* (2008) found that Eucalyptus oil possesses a wide spectrum of biological activity including anti- microbial, fungicidal, insecticidal/ insect repellent, herbicidal, acaricidal and nematicidal. Pest control can be achieved using eucalyptus oils against bacteria, fungi, insects, nematodes, weeds and mites. The use of eucalyptus oil as a natural pesticide is of immense significance in consideration of the environmental and toxicological implications of the indiscriminate use of synthetic pesticides.

Cloyd (2008) illustrated that the fungus gnat management requires a holistic approach based on implementation of cultural (sanitation and water management), chemical (microbial insecticides and insect growth regulators) and biological control (predatory mites, beetles and entomopathogenic nematodes). He explained that scouting for the gnat involves the use of yellow sticky cards for adults and potato disks for the larvae.

Park *et al.* (2008) tested essential oils from 20 plant species for their insecticidal activity against larvae of *Lycoriella ingenua* (Dufour) (Diptera: Sciaridae) by using a fumigation bioassay. Among them, caraway seed, spearmint, cumin, and thyme red essential oils were highly effective against *L. ingenua* at 20 X 10^{-3} mg/ml air. Effects of four selected plant essential oils on growth of oyster mushroom, *Pleurotus ostreatus*, were also investigated.

Shamshad *et al.* (2008) tested the toxicity of six commercial formulations of insecticides or bio pesticides against third larval instar of *Lycoriella ingenua* by using laboratory bioassays. Out of these, triflumuron was the most effective insecticide against *L. ingenua* with 93 per cent mortality followed by diazinon with 90 per cent mortality. Abamectin and *Bacillus thuringiensis* var. *israelensis* were found ineffective. *Steinernema feltiae*, an entomopathogenic nematode, applied at higher rates gives 93.33 per cent mortality.

Akob and Ewete (2009) performed laboratory studies on contact toxicity and repellency against *Sitophilus zeamais* with ethanolic extracts of *Eucalyptus grandis* leaves, *Vetiveria zizanioides* roots, *Cupressus arizonica*, and *Ocimum gratissimum*,. The ethanolic extracts of all

the four plants caused mortality and showed repellency to great extent depending upon the concentration of the extract.

Erler *et al.* (2009) concluded that insect pests of mushroom production are cecidomyiid, sciarid and phorid flies with *Megaselia halterata* (Wood) (Diptera: Phoridae) being the most common species. In their study, two commercial microbial products (a bacterial larvicide, *Bacillus thuringiensis* var. *israelensis* Berliner (*Bti*) and an entomopathogenic nematode, *Steinernema feltiae* (Filipjev) and spinosad, a biologically- derived insecticide were evaluated for control of *M. halterata*. Treatments with the microbial products had significantly lower numbers of adult emergence 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. 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 water treated control. These results suggested that these microbial products can be used as alternatives to conventional chemicals in controlling M. *halterata* on mushroom.

Nerio *et al.* (2009) isolated essential oils from *Cymbopogon citratus* and *Eucalyptus citriodora* in Colombia and analysed them by gas chromatography– mass spectrometry (GC-MS) and tested for repellent activity and contact toxicity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). The main components of *C. citratus* oil were geranial (34.4 per cent), neral (28.4 per cent) and geraniol (11.5 per cent), whereas those of *E. citriodora* were citronellal (40 per cent), isopulegol (14.6 percent) and citronellol (13 per cent). These studies showed the composition and repellent activity of essential oils, suggesting that these are potential candidates as insect repellents.

Shamshad (2010) described *Bradysia ocellaris* and *Lycoriella ingenua* (Diptera: Sciaridae) as major pests of cultivated mushrooms, *Agaricus bisporus* (Lange) Imbach. Mushrooms are susceptible to a large range of diseases and pests that can cause serious crop loss. These pests cause losses in yield through larval damage of the compost, mycelium and sporophores. Adults of these flies also act as vectors for mites and fungal diseases in cultivated mushrooms. Integrated pest management in mushrooms is dependent upon four main principals of sanitation, exclusion, monitoring and pest control.

Farsani *et al.* (2011) studied the toxicity of aqueous extracts of two plants, *Nicotiana tobacum* and *Eucalyptus globulus* against second instar larvae of *Lycoriella auripila* (Diptera: Sciaridae), using agar dilution technique. Seven concentrations of aqueous extracts of both plants were applied on second instar larvae and their mortality were evaluated after 24, 48 and 72 h. The obtained results revealed that aqueous extracts of *N. tabacum* and *E. globulus* caused 77.55 and 72.5 per cent mortality of larvae of *L. auripila* at concentration of 4000 ppm after 72 h, respectively. The estimated LC_{50} after 24, 48 and 72 h were 7316.5, 2468.5 and 2013.1 ppm for tobacco and 64870.0, 6839.5 and 3326.4 ppm for eucalyptus, respectively.

Ebadollahi *et al.* (2013) tested the larvicidal activity of leaf essential oils from three eucalyptus species (*Eucalyptus largiflorens*, *Eucalyptus oleosa* and *Eucalyptus spathulata*) against American white moth, *Hyphantria cunea* (Lepidoptera: Arctiidae). Mortality was recorded daily for three days after treatment. Leaf disc bioassays revealed that all three oils had strong insecticidal activity as LC_{50} for *E. oleosa*, *E. spathulata*, and *E. largiflorens* at 24 h exposure time were 0.36, 0.61, and 1.24%, respectively. The time needed to kill 50% values were calculated as 9.09 h with *E. largiflorens*, 11.03 h with *E. oleosa*, and 13.03 h for *E. spathulata*. Based on probit analysis, an increase in the susceptibility of the insect was associated with an increase in the different concentrations of all oils and the increase in the time of exposure.

Mohammed (2013) illustrated repellency of ethanolic extracts of five plants extract *viz.* Euc*alyptus gluaca*, *Melia azedarach*, *Mentha arvensis*, *Olea europaea* leaves and pericarp of *Punica granatum* against adult and larvae of the confused flour beetle *Tribolium confusum* . The plant extracts were applied at four concentrations (2.5%, 5%, 7.5% and 10%) for each adult and larvae. All the plant extracts were found to be effective repellents and showed more than 20 % average repellency. For larval stage it was observed that *E. glauaca* induce 100% repellency with concentration 7.5% at 2h after exposure.

Nair *et al.* (2014) investigated the larvicidal activity of different solvent leaf extracts (hexane, diethyl ether, dichloromethane, and methanol) of *Eucalyptus globulus* and *Centella asiatica* against two geographically different strains of *Aedes aegypti* and *Anopheles stephensi.* Larval mortality was observed after 24 hours of treatment. LC_{50} and LC_{90} were calculated. The

hexane extracts of both plants and the diethyl ether extract of *C. asiatica* presented the highest potential for the control of *Aedes aegypti* and *Anopheles stephensi*.

Cloyd (2015) described cultural methods, physical methods and sanitation for the management of fungal gnat which includes elimination of algae, plant and growing medium debris, placing physical barriers onto the growing medium surface and using different types of materials that repel fungus gnat adults.

Sahin *et al.* (2016) carried out a study in two successive mushroom growing periods in 2014 to determine the effectiveness of three different coloured (yellow, blue and white) sticky traps in capturing mushroom flies; mushroom phorid fly *Megaselia halterata* Wood (Diptera: Phoridae), mushroom sciarid fly *Lycoriella ingenua* (Dufour) (Diptera: Sciaridae) and mushroom scatopsids *Scatopse* spp. (Diptera: Scatopsidae). The traps were replaced with fresh ones at weekly intervals. The results showed that yellow traps captured significantly more adults of *L. ingenua* than blue and white traps, when tested at weekly intervals. There were no significant differences between yellow and blue traps in terms of adult catches of *M. halterata* and *Scatopse* spp. White traps were the least effective in capturing all fly species as compared to other traps tested in both periods. Overall results indicate that yellow and blue colors can be used to enhance the effectiveness of sticky traps in capturing the mushroom flies.

Said *et al.* (2016) elucidated the effect of trap color and height for Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). The results showed that yellow trap was consistently the most attractive trap amongst the other trap colors tested with an overall average of 62.6 adults per trap during the study. The second most attractive traps were white and green traps with overall averages of 45.2 and 40 adults per trap, respectively. The attractiveness levels of the red and blue traps were comparable to each other with overall averages of 29.4 and 25.4 adults per trap, respectively. The least attractive trap was black trap with an overall average of 17.1 adults per trap, which was significantly lower than the other trap colors.

Badaki *et al*. (2018) revealed that the ethanolic extracts of the plant recorded higher percentage of larvae mortality than the aqueous extracts. Ethanolic plant extracts also recorded very low LC_{50} and LC_{90} values unlike the aqueous extracts, indicating high larvae toxicity. Their findings indicated that ethanolic plant extracts had very high lethal effect on *Aedes* larvae

compared to their aqueous extracts. Although, the ethanolic extract of *Ocimum gratissimum* alone was more potent than *Eucalyptus camaldulensis* than their combined formulation. The mortality of *Aedes* larvae was dose and time-dependent with increase in larvae mortality being directly proportional to time period of exposure of the larvae to the plant extracts.

Mazin *et al.* (2019) studied the activity of mushroom phorid fly (*Megaselia halterata*) by placing traps at different locations in and outside the mushroom houses. In the turf areas the activity was found to be higher than wind breaks and at higher temperature activity was also high but it ceased when temperature went down.

Chapter -3

MATERIALS AND METHODS

The research work on **"Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)"** was carried out in the laboratory of Department of Entomology in College of Horticulture and Forestry Neri, Hamirpur (H.P), during 2018-2020 using the following "Materials and Methods":

3.1 Incidence of dipteran flies on oyster mushroom (*Pleurotus* spp.)

Keeping in view all the geographical parameters like elevation, longitude, latitude etc. different mushroom farms were sampled randomly in the districts of Hamirpur, Kullu and Kangra in Himachal Pradesh. A sample of compost and fruiting bodies during cropping was collected during the survey studies. The insects and their post embryonic stages were separated manually. Different stages of the insect pests recorded during survey was preserved in 70.0 per cent alcohol. Incidence of dipteran flies on oyster mushroom farms was calculated as:

$$\text{Pest incidence (\%)} = \frac{Number\ of\ infested\ bags}{Total\ number\ of\ bags} \times 100$$

13.1.1 Identification of dipteran flies on oyster mushroom (*Pleurotus* spp.)

All the dipteran flies collected during survey studies from different mushroom farms were photographed and identified with the help of published literature and taxonomic keys. Specimens were also sent to ZSI, Kolkata for confirmation of the specimens.

13.1.2 Maintenance of mushroom flies culture in laboratory

The population of different mushroom flies encountered during survey were collected and maintained in the laboratory by keeping them in plastic cages by providing 500.0 g insect pest free spawned mushroom compost and the culture was used for further experiments.

13.2 Biology of mushroom flies on oyster mushroom (*Pleurotus* spp.)

Biology of two major dipteran flies collected during the survey was studied in the laboratory.

Observations recorded:

i. Fecundity rate- Freshly emerged one pair of adult fly (male and female) was released in the insect cage having fresh spawned compost in Petri plate. The same set was replicated thrice. No. of eggs were counted every day and fresh spawned compost was provided everyday till the female fly stopped egg laying.

ii. Pre-oviposition period- Time of emergence of adult female fly from pupa was recorded and after emergence the female fly was transferred to the insect cage and was provided with Petri plate having fresh insect free spawned compost. The same set was replicated thrice. The female fly was observed for egg laying at 12 hours interval till the egg laying started.

iii. Oviposition period- The time period from first to last egg laying was recorded. Petri plates for observation were replaced every day and were replicated thrice.

iv. Incubation period- Days from egg laying to hatching were counted. On an average 20 eggs were taken for each observation.

v. Larval period- The time period from 1st to last instar was recorded.

vi. Pupal period- The time period from pupa formed to emergence of adult was recorded.

vii. Adult longevity-The time period for which the adult remained alive was recorded.

viii. Male: female ratio- 20 pupae were kept in petri plate and proper moisture was maintained. Males and females emerged were counted randomly.

ix. Total life cycle- The total time taken from egg to adult emergence was recorded.

3.3 MANAGEMENT

3.3.1 Evaluation of botanicals in oyster mushroom using different formulation for their insecticidal property against insect pests.

Aqueous**,** ethanolic and diethyl ether leaf extract of *Eucalyptus globulus* were tested for their insecticidal efficacy against insect pests of oyster mushroom.

3.3.2 Collection of plant leaves for extraction

Leaves of eucalyptus plant were collected from the surroundings of Hamirpur (H.P.) and were dried under shade before being pounded with a wooden stick to make a coarse powder (Plate 1) and were stored in a cool and dry place in the room.

Plate 1 Eucalyptus leaf powder

(a) **(b)** **(c)**

Plate 2 Eucalyptus leaf extracts (a) aqueous (b) ethanolic (c) diethyl ether

3.3.3 Preparation of aqueous, ethanolic and diethyl ether leaf extract

Shade dried leaves were powdered with the help of electric grinder. A sample of 20.0 g powder was weighed and soaked in 200.0 ml distilled water / ethanol / diethyl ether overnight in a beaker (Plate 2). Solvents were evaporated and extract was further dried. The extract obtained after drying was again dissolved in 200.0 ml distilled water and the contents were filtered through the Whattman No. 1 filter paper. This stock solution was designated as 10.0 per cent leaf extract and was used for further experiments.

3.3.4 Preparation of doses

Ten per cent concentration of leaf extract of *E. globulus* was prepared using water, ethanol and diethyl ether as extraction solvents at an ambient room temperature and was then filtered through Whattman No. 1 filter paper. Different concentrations were prepared from the stock solutions for aqueous, ethanolic and diethyl ether leaf extracts. Preliminary standardization of the doses was carried out by calculating LC_{50} and LC_{90} in the laboratory for evaluating their effect on per cent larval mortality and per cent mycelial inhibition. LC_{50} and LC_{90} were calculated against the mushroom fly by taking log values of the concentration and probit values of larval mortality and then plotting it on a graph (Finney, 1971).

13.3.5 Preparation of Agniastra

Composition-

S. No.	Ingredients	Composition
1.	Green chillies	50.0 g
2.	Garlic	50.0 g
3.	Neem leaves	500.0 g
4.	Cow urine	1500.0 ml
5.	Tobacco leaves	50.0 g

Five ingredients were used to prepare 2.0 litres of agniastra solution (Plate 3). The ingredients were ground into fine paste and were then mixed with cow urine. This solution was boiled four times

and then left for 48 h, filtered and finally some water was added and it was designated as agniastra 2.0 per cent.

3.3.6 Preparation of Malt extract agar medium

The flies were reared on artificial media using malt extract.

Composition-

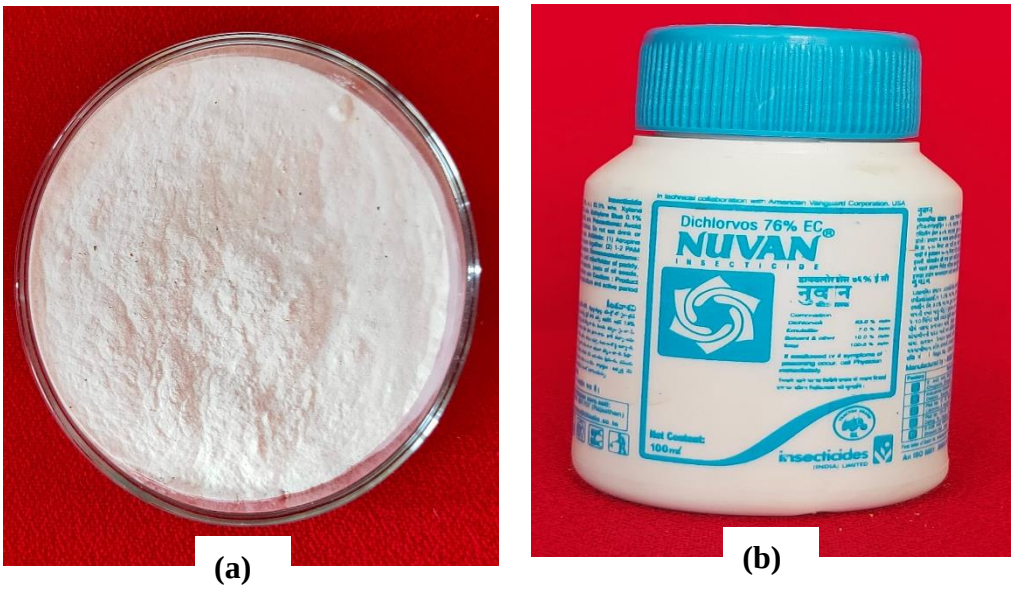
S. No.	Ingredients	Composition
1.	Malt extract	25.0 g
2.	Agar- agar	20.0 g
3.	Distilled water	1000.0 ml

Method of preparation

i. The medium was prepared by melting 25.0 g of malt extract in 500.0 ml of distilled water by heating.
ii. 20.0 g of agar-agar was added with continuous stirring till all the constituents were thoroughly mixed up and final volume was made upto 1000.0 ml.
iii. pH of the medium was adjusted to 7.
iv. The medium so prepared was poured in conical flasks and these were plugged tightly with non-absorbent cotton and autoclaved at 15 psi for 20 minutes.
v. The medium thus prepared was poured in sterilized Petri plates under aseptic conditions (using the sterilized laminar flow air chamber) and allowed to solidify.

By using poison food technique (Falck, 1907) different concentrations of aqueous/ ethanolic/ diethyl ether extracts of *Eucalyptus globulus* were mixed with the agar media. The effect of botanicals on mycelial inhibition was estimated and larval mortality was checked by allowing the larvae to feed on these Petri plates.

Plate 3 Preparation of Agniastra (2%)



(a) **(b)**

Plate 4 Formulations used (a) *Bacillus thuringiensis* (b) Nuvan

3.3.7 To study the effect of *Bacillus thuringiensis* against most prevalent insect pests of oyster.

B. thuringiensis was used at different concentration to control the insect pests (Plate 4). Effect on per cent larval mortality and per cent larval mycelial inhibition was evaluated.

Percent larval mortality = $\frac{Number\ of\ dead\ larvae}{Total\ number\ of\ larvae} \times 100$

Percent mycelial inhibition = $\frac{C-T}{C} \times 100$

Where,

C= Colony diameter in control

T= Colony diameter in treatment

3.3.8 Evaluation of different coloured sticky traps against mushroom flies associated with oyster mushroom.

Double sided different coloured sticky traps (10×20 cm) *viz.* yellow, blue, green, red and white with bulbs (0.5 Watt) and without bulbs were placed inside the cages. The traps were divided into squares and coated with mustard oil. Adult flies were introduced into the cage.

Colour of the trap preferred by the pest was noted down and number of insects that were found sticking to the trap in each square was calculated.

13.3.9 To study the repellent effect of different eucalyptus formulations.

100 ml capacity beakers were filled with spawned compost. In total twelve beakers having compost were placed in the insect cage. Out of twelve, nine beakers treated with the 10.0 per cent concentration of aqueous/ ethanolic/ diethyl ether leaf extracts of eucalyptus were placed randomly in the insect cage. Three sets of replication were made for observation in the laboratory. Twenty number of adult of *Bradysia asiatica* were released in the cage having beakers. The observations were recorded after 24 hours and the number of flies landed on the compost kept in the beaker were counted.

Percent repellency= $\frac{UT-T}{UT} \times 100$

Where,

T = average number of flies on treated beakers

UT = average number of flies on untreated beakers

13.3.10 Statistical analysis

Results were statistically analyzed by using Completely Randomized Design (CRD) for laboratory experiments (Panse and Sukhatme, 2000). Critical difference at 5.0 per cent level of significance was calculated to find out the significance of the results obtained. LC_{50} and LC_{90} were also evaluated using Probit analysis.

Chapter- 4

RESULTS AND DISCUSSION

Present study was conducted with the objectives to record the incidence of insect pests in different mushroom units of Himachal Pradesh along with the biology and bio-intensive methods of management for the most prevalent insect pest recorded during the survey. Studies were also carried out to test the efficacy of different concentrations of aqueous, diethyl ether and ethanolic extracts of *Eucalyptus globulus*, agniastra and microbial pesticides (*Bacillus thuringiensis*) on larval mortality as well as mushroom mycelial inhibition. Repellent property of eucalyptus extracts was inspected against adult flies. Different coloured sticky traps with and without bulbs were used to check the preference of trap color by the flies.

4.1 Incidence of dipteran flies on oyster mushroom (*Pleurotus* spp.)

Ten mushroom farms were surveyed randomly in the districts of Hamirpur, Kullu and Kangra. A sample of compost and fruiting bodies was collected during the survey studies. Yellow sticky traps were used to collect adult flies and a sample of compost was taken at spawn run and cropping stage. The flies were small, thin and mosquito-like and damaged sporophores and also acted as vectors of mites, nematodes, bacterial and fungal pathogens. They deposited their eggs on the gills, stipe and compost mycelium. Larvae were seen feeding on the compost mycelium and the fruiting bodies resulting in tunneling. Data on mushroom fly incidence is presented in Table 1.

From the data it is delineated that the fly incidence was highest in Hamirpur (62.86%) followed by Kullu (51.33%) and Kangra (42.70%) as high temperature in district Hamirpur promotes greater activity of mushroom flies. The infestation of dipteran flies ranged from 38.46 to 66.67 per cent on *Pleurotus* spp. in different locations. The overall incidence of the dipteran flies in three districts was 52.55 per cent. Sciarid flies were more prevalent in all mushroom farms surveyed in comparison to the phorid flies. The bags that were infested with flies did not produce quality mushrooms making them unfit for consumption and marketing.

Table 1. Incidence of dipteran flies on *Pleurotus* spp. at different mushroom units of Himachal Pradesh

Location	Units surveyed	Total bags cultivated	Infested bags	Per cent incidence	Mean fly incidence (%)	Overall Mean (%)
Hamirpur	Kangoo	400	230	57.50	62.86	52.55
	Bamnoh	250	154	61.60		
	Nadaun	300	197	65.67		
	Putriyal	300	200	66.67		
Kullu	Dughilag	500	280	56.00	51.33	
	Bandrol	300	140	46.67		
Kangra	Palampur	450	200	44.44	42.70	
	Baijnath	200	87	43.50		
	Thera	350	136	38.86		
	Majhin	250	110	44.00		

The present studies are in agreement to some earlier studies where sciarid and phorid flies have been reported as most serious pests of oyster mushroom. Gnaneswaran and Wijayagunasekara, 1999 performed a similar survey and identified insect pests of oyster mushroom in central province of Sri Lanka. They also reported a damage of 82 per cent was caused to the crop by the flies. Nongkynrih *et al*., 2017 in a similar survey in Northeast India during 2013- 2014 and identified various arthropod pests *viz*., sciarid fly (*Bradysia* spp.), pleasing fungus beetles, mycetophilid fly, fruit fly and mites infesting oyster mushroom leading to yield loss. Pest incidence was found up to 100 per cent during the rainy season (May, June, July and August). Joshi, 2009 also screened six species of *Pleurotus*, during February-March (2008 and 2009) and found high and moderate infestation by phorid and sciarid flies, respectively.

4.1.1 Identification of dipteran flies

The insects and their post embryonic stages collected during the survey were separated manually in vials containing 70.0 per cent alcohol following Lewandowski *et al*., 2004.

All the species of oyster mushroom flies were photographed and about 20-25 number of adult flies (male/ female), larvae and pupae collected in vials were sent to ZSI, Kolkata for

identification. Their important features were studied and compared with the published literature and taxonomic keys.

Table 2. List of flies identified from Zoological Survey of India, Kolkata

Location	Units surveyed	Species identified by ZSI	Family	Order	ZSI Lot No.	Identification Report No.	Appendix
Kullu	Dughilag	*Bradysia asiatica*	Sciaridae	Diptera	58/2019	11/2019	III
	Dughilag	*Megaselia* sp.	Phoridae	Diptera	58/2019	11/2019	
	Bandrol	*Sciara* sp.	Sciaridae	Diptera	40/2020	06/2020	
Hamirpur	Kangoo	*Sciara orientalis*	Sciaridae	Diptera	02/2020	01/2020	

Table 2 depicts the species collected from different mushroom units and identified from ZSI, Kolkata. A mixed culture of flies (*Bradysia asiatica* and *Megaselia* sp.) was present from the samples collected from Dughilag, Kullu while *Sciara* sp. was identified from another sample collected from Bandrol, Kullu and *Sciara orientalis* was the species identified from Kangoo, Hamirpur.

4.2 Biology of *Bradysia asiatica* and *Sciara* sp. on oyster mushroom (*Pleurotus* spp.)

The life cycle of *B. asiatica* and *Sciara* sp. was studied under the laboratory conditions at COHF, Neri. Three sets of replications were maintained for each species. Data on the life history is presented in Table 3 and 4.

4.2.1 Fecundity rate: Freshly emerged one pair of adult fly (male and female) was released in the insect cage having fresh compost in Petri plate. Number of eggs were counted every day and fresh compost was provided everyday till the female fly stopped laying eggs. The average fecundity rate for *B. asiatica* was 21.67 ± 2.03 eggs and for the *Sciara* sp. it was 28.67 ± 2.03 eggs.

4.2.2 Pre-oviposition period: Time of emergence of adult female fly from pupa was recorded and after emergence the female fly was transferred to the insect cage and was provided with Petri plate having fresh insect free compost. The female fly was observed for egg laying till the egg laying

started. The pre-ovipositional period for *B. asiatica* and *Sciara* sp. was 1.67 ± 0.33 and 1.33 ± 0.33 days, respectively.

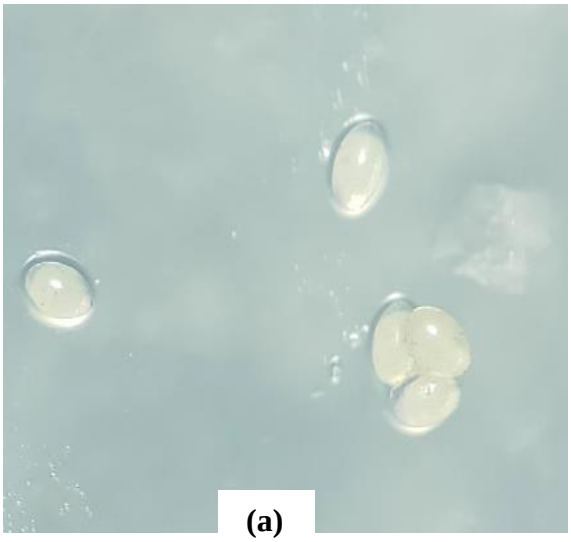
4.2.3 Oviposition period: The time period from first to last egg laying was recorded. Petri plates for observation were replaced every day and the oviposition period for *B. asiatica* and *Sciara* sp. for 3 ± 0.58 and 3.33 ± 0.33 days, respectively.

4.2.4 Incubation period: The eggs of *B. asiatica* (Plate 5) were oval, smooth, shiny and translucent and were laid singly or in small clusters of 3-4 eggs while the eggs of *Sciara* sp. (Plate 6) were oval, dirty white and semi-transparent in color that turned transparent towards the time of hatching. Eggs were laid singly as well as in groups selectively on the mycelium. The incubation period was 3.33 ± 0.33 and 2.33 ± 0.33 days for *B. asiatica* and *Sciara* sp., respectively. Similar observations about the eggs and incubation period were made by Katumanyane *et al.*, 2018 for *Bradysia* spp. (Diptera: Sciaridae).

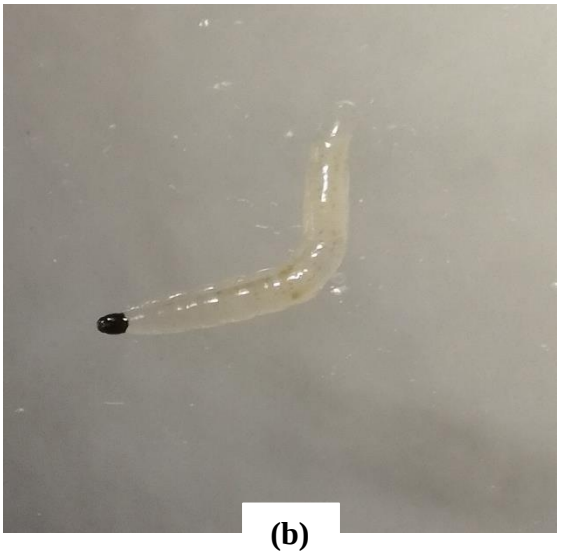
Table 3. Biology of *Bradysia asiatica* on oyster mushroom (*Pleurotus* spp.)

Developmental stage	Mean	S.E.
Ovipositional period (days)	3.00	0.58
Pre-oviposition period (days)	1.67	0.33
Incubation period (days)	3.33	0.33
Larval period (days)	10.67	0.33
Pupal period (days)	4.33	0.33
Adult longevity (days)	4.67	0.33
Total life cycle (days)	23.00	1.00
C.D. SE(m) SE(d) C.V.		1.59 0.52 0.73 12.43

4.2.5 Larval period: The larvae were apodous, slender with strongly sclerotized head capsule of black to brown color and translucent body. Their skin was semi-transparent which revealed all the contents of the digestive tract. The larvae fed on the mycelium agar plate and went through four



(a)



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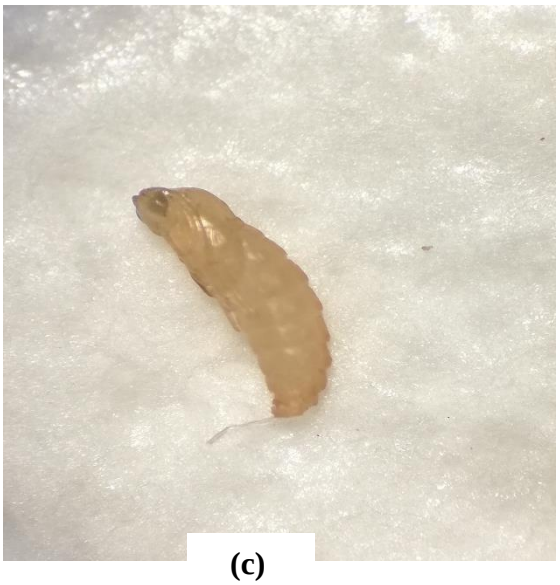

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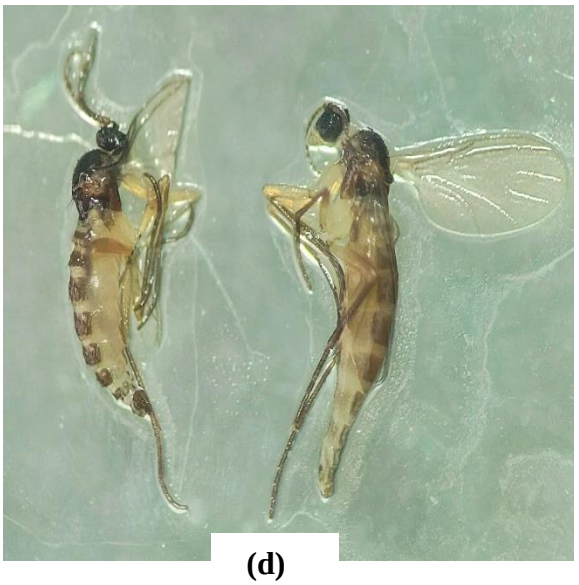
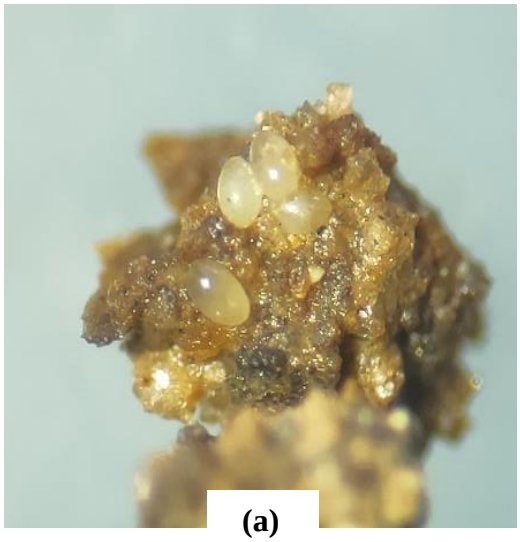
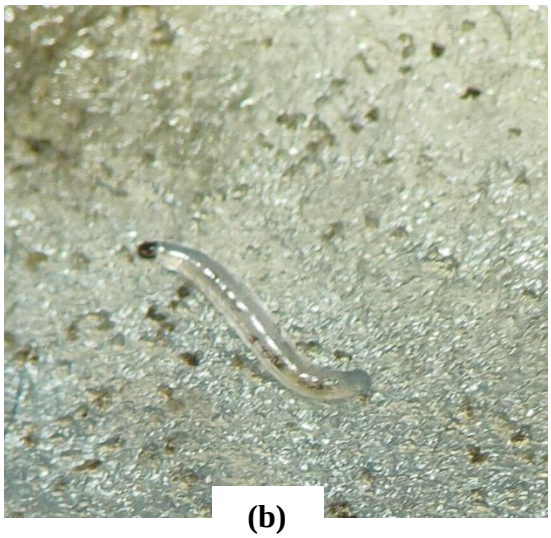


(d)

Plate 5 Different stages of *Bradysia asiatica* (a) Eggs (b) Larva (c) Pupa (d) Adults



(a)



(b)

(c)

(d)

Plate 6 Different stages of *Sciara* sp. (a) Eggs (b) Larva (c) Pupa (d) Adults

developmental stages before turning into a pupa. Initially the larvae were too small but with every moult, the instar increased in length. The time taken by both the species was 10.67 ± 0.33 days. Similar results were recorded by Katumanyane *et al.*, 2018 for *Bradysia coprophila* and *Bradysia impatiens*. Choi *et al*., 2000 also recorded mean larval period of 10.5 days at 28°C for *Coboldia fuscipes* (Diptera: Scatopsidae).

4.2.6 Pre-pupal and Pupal period: The fully grown larva after completing its development stopped feeding for some time and became sluggish. It moved to a certain depth into mycelial threads and spun a cocoon for pupation. The newly formed pupa was white which later turned yellow and finally to golden brown color. The pupa had visible appendages that were close to the body and resembled the adult fly.

Table 4. Biology of *Sciara* sp. on oyster mushroom (*Pleurotus* spp.)

Developmental stage	Mean	S.E.
Pre-oviposition period (days)	1.33	0.33
Ovipositional period (days)	3.33	0.33
Incubation period (days)	2.33	0.33
Larval period (days)	10.67	0.33
Pupal period (days)	4.33	0.33
Adult longevity (days)	4.00	0.58
Total life cycle (days)	21.33	0.67
C.D. SE(m) SE(d) C.V.		1.34 0.44 0.62 11.18

The pupal period was completed in a lapse of 4.33 ± 0.33 days by both the species. Lee *et al*., 2010 reported that the growth period of pupa lasts about 5 days depending on the prevailing temperature. However, the variation in duration is relatively due to the temperature, with it being mostly about 3 days.

4.2.7 Adult longevity: The adults that emerged from the pupa were small, brown colored flies with prominent eyes, transparent wings and thread like antennae. Adults did not feed on the mycelium.

The females were longer than the males and had an ovipositor at the end. The males possessed a pair of claspers for holding the female during mating. The duration from the date of emergence to death of adults was considered as the adult longevity. The longevity period was 4.67 ± 0.33 and 4 ± 0.58 days for *B. asiatica* and *Sciara* sp., respectively. Katumanyane *et al.*, 2018 reported adult longevity of 4-7 days and longevity of males was short as compared to females.

4.2.8 Male: female ratio: 20 pupae were kept in Petri plate and proper moisture was maintained. Males and females emerged were collected and counted. The ratio was 6.66: 13.33 for *B. asiatica* and 7.5: 12.5 for *Sciara* sp. Nigro *et al.*, 2007 found that the sex-ratio of *Sciara ocellaris* was symmetrical (*i.e.* about 50% males) at 18-20°C while at the higher temperatures of 24-28°C the distributions were skewed towards a high proportion of females with the mean proportion of males decreasing to about 30-37 per cent.

4.2.9 Total life cycle: The total time taken from egg laying to adult emergence for *B. asiatica* and *Sciara* sp. was 23 ± 1 and 21.33 ± 0.67 days, respectively. Choi *et al*., 2000 reported that the total time period for complete development for *Coboldia fuscipes* is dependent on the temperature and varied as 24.5 and 18.9 days at 25 and 28°C, respectively.

4.3.1 Evaluation of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on the growth of mushroom mycelia of *Pleurotus* spp.

Aqueous (5.0, 7.0, 10.0%)**,** ethanolic (7.0, 9.0, 10.0%) and diethyl ether (8.0, 9.0, 10.0%) extracts of *E. globulus*, agniastra (2.0%) and *B. thuringiensis* (0.0001 and 0.0005 %) were prepared and mixed with the agar before pouring. The inhibitory effect of different extracts were checked on the mushroom mycelium of *Pleurotus* spp. by comparing them with the control.

It can be perceived from Table 5 that there was complete mycelial inhibition (100.00%) for eucalyptus leaf ethanolic extracts at all concentrations (Plate 7). The inhibitory effect of *B. thuringiensis* (0.0001%, Plate 8) was 34.17 per cent while for 10.0 per cent diethyl ether extract, it was 21.90 per cent. No mycelial inhibition was shown by agniastra (2.0%, Plate 9), aqueous extracts of *E. globulus* and 8.0 per cent diethyl ether leaf extract of eucalyptus.

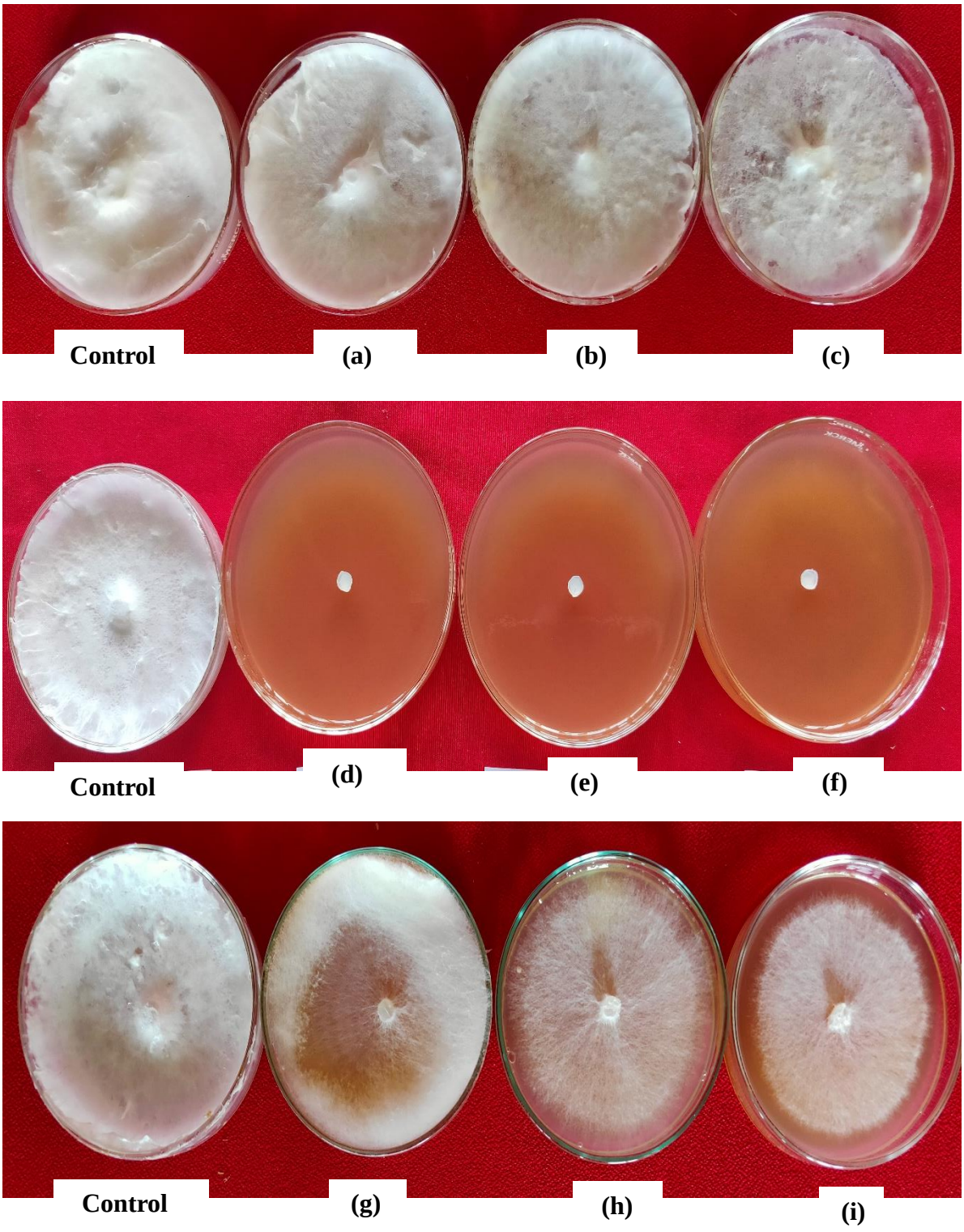


Plate 7 Mycelial inhibition shown by eucalyptus leaf extracts (a) aqueous 5% (b) aqueous 7% (c) aqueous 10% (d) ethanolic 7% (e) ethanolic 9% (f) ethanolic 10% (g) diethyl ether 8% (h) diethyl ether 9% (i) diethyl ether 10%

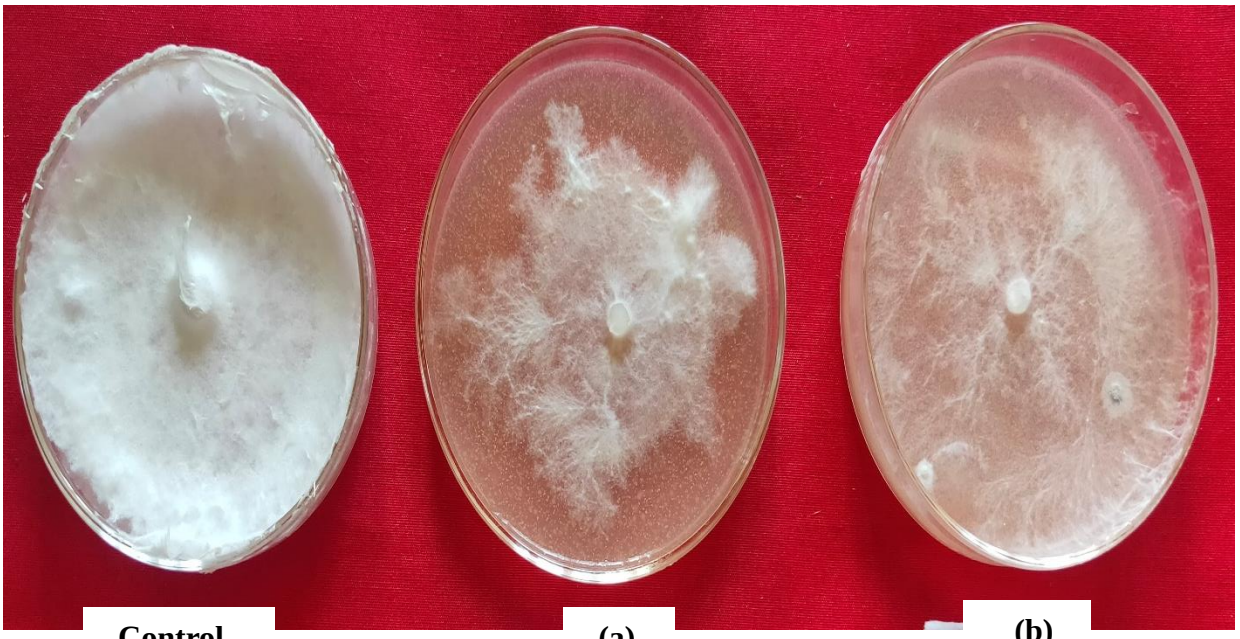


Plate 8 Mycelial inhibition shown by *Bacillus thuringiensis* (a) 0.0001% (b) 0.0005%

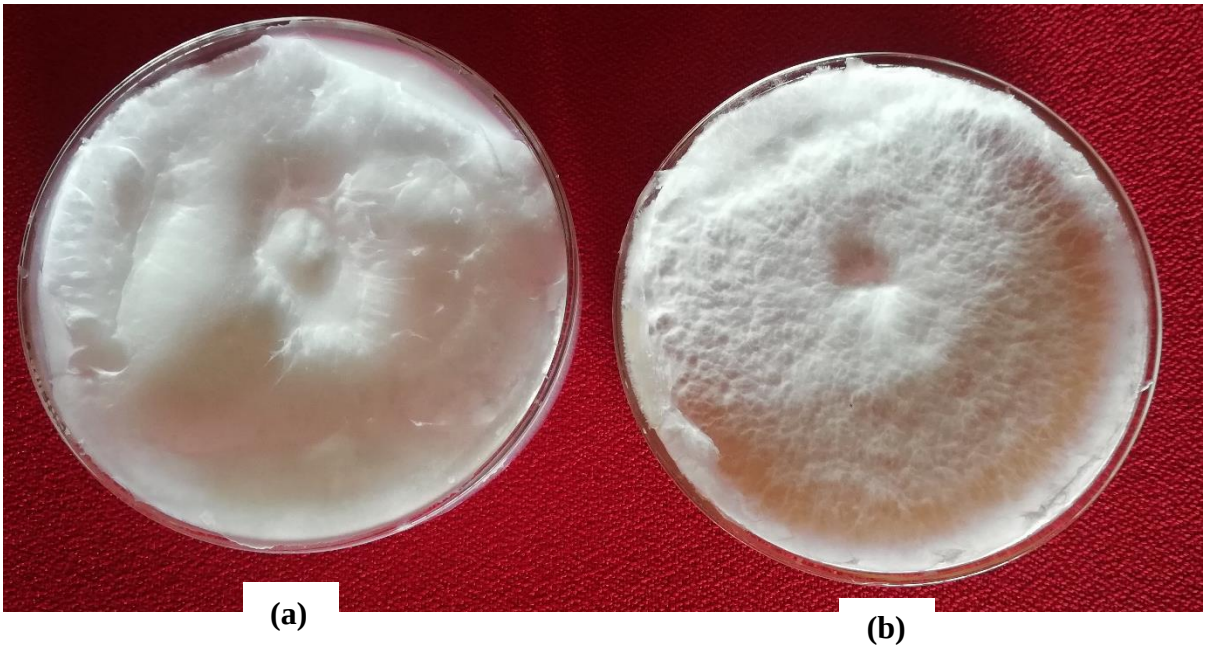


Plate 9 Mycelial inhibition shown by agniastra (a) control (b) agniastra 2%

Table 5. Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on the growth of mushroom mycelia of *Pleurotus* spp.

Treatment	Conc. (%)	Mycelial inhibition (%)
Aqueous leaf extract of *E. globulus*	5.0	0.00 (1.00)
Aqueous leaf extract of *E. globulus*	7.0	0.00 (1.00)
Aqueous leaf extract of *E. globulus*	10.0	0.00 (1.00)
Diethyl ether leaf extract of *E. globulus*	8.0	0.00 (1.00)
Diethyl ether leaf extract of *E. globulus*	9.0	16.66 (4.20)
Diethyl ether leaf extract of *E. globulus*	10.0	21.91 (4.79)
Ethanolic leaf extract of *E. globulus*	7.0	100.00 (10.05)
Ethanolic leaf extract of *E. globulus*	9.0	100.00 (10.05)
Ethanolic leaf extract of *E. globulus*	10.0	100.00 (10.05)
B. thuringiensis	0.0001	34.17 (5.93)
B. thuringiensis	0.0005	5.42 (2.53)
Agniastra	2.0	0.00 (1.00)
Untreated control		0.00 (1.00)
C.D.		0.09
SE(m)		0.03
SE(d)		0.05
C.V.		1.35

Figures in the parentheses are square root transformed values

4.3.2 Bio-efficacy of leaf extracts of *E. globulus*, agniastra and *B. thuringensis* against larval mortality of *B. asiatica* and *Sciara* sp.

Cultures of flies were reared and maintained in the laboratory and were used for the experiment. Ten larvae which were at 2nd and 3rd larval instar stage were collected and transferred to Petri plate containing mycelium of *Pleurotus* spp. They were allowed to feed on the plate that

was prepared by mixing the plant extracts, agniastra and microbial pesticide into the agar plate, separately. As 100.00 per cent mycelial inhibition was shown by ethanolic extracts of *E. globulus*, therefore, larval mortality of ethanolic extract was not tested against *B. asiatica* and *Sciara* sp. Larval mortality was considered at an exposure periods of 24 and 48 h.

Table 6. Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on larval mortality of *B. asiatica* at different exposure periods

Treatment	Conc. (%)	Per cent larval mortality at different exposure period		Mean
		24 h	48 h	
Aqueous leaf extract of *E. globulus*	5.0	26.67 (30.98)	43.33 (41.14)	35.00 (36.06)
Aqueous leaf extract of *E. globulus*	7.0	53.33 (46.90)	70 (56.97)	61.67 (51.94)
Aqueous leaf extract of *E. globulus*	10.0	73.33 (58.98)	86.67 (68.83)	80.00 (63.90)
Diethyl ether leaf extract of *E. globulus*	8.0	33.33 (35.20)	46.67 (43.06)	40.00 (39.13)
Diethyl ether leaf extract of *E. globulus*	9.0	43.33 (41.05)	66.67 (54.97)	55.00 (48.01)
Diethyl ether leaf extract of *E. globulus*	10.0	66.67 (54.76)	73.33 (58.98)	70.00 (56.87)
Agniastra	2.0	66.67 (54.76)	83.33 (66.12)	75.00 (60.44)
B. thuringiensis	0.0001	26.67 (30.98)	43.33 (41.14)	35.00 (36.06)
B. thuringiensis	0.0005	36.67 (37.21)	53.33 (46.90)	45.00 (42.06)
Nuvan	0.1	83.33 (66.12)	90.00 (71.54)	86.67 (68.83)
Untreated control		0	0	0
Mean		46.36 (41.54)	59.69 (49.97)	
$CD_{0.05}$		Treatment (T)		7.87
		Exposure period (P)		3.36
		T X P		-

Figures in the parentheses are angular transformed values

A perusal of data in Table 6 for management of *B. asiatica* indicated that the mean larval mortality was significantly higher at an exposure period of 48 h (59.69%) in comparison to 24 h

(46.36%). The highest mortality was 80.00 per cent that was achieved at 10.0 per cent concentration of aqueous leaf extract of eucalyptus followed by 75.00 per cent mortality of agniastra (2.0%) though being significantly lower than standard check nuvan (86.67%).

The lowest larval mortality of 35.00 per cent was observed for *B. thuringiensis* (0.0001%) and 5.0 per cent aqueous leaf extract of eucalyptus. Mean per cent mortality increased with the increase in concentration of particular extract and exposure time. At 48 h exposure period maximum larval mortality was shown by 10.0 per cent aqueous extract (86.67%) followed by 2.0 per cent agniastra (83.33%) and 10 per cent diethyl ether leaf extract (73.33%). The lowest larval mortality was attained by *B. thuringiensis* (0.0001%) and 5.0 per cent aqueous leaf extract (43.33%) at 48 h exposure period. Similar trends were observed at 24 h exposure period with maximum larval mortality at 10.0 per cent aqueous leaf extract (73.33%) followed by 2.0 per cent agniastra and 10.0 per cent diethyl ether leaf extract (66.67%) and the lowest by *B. thuringiensis* (0.0001%) and 5.0 per cent aqueous leaf extract (26.67%).

The results presented in Table 7 for the management of *Sciara* sp. followed similar trends to that of *B. asiatica* with maximum mean mortality at 48 h (58.48%) followed by 24 h (43.03%). The highest mortality was attained at 10.0 per cent concentration of eucalyptus aqueous leaf extract (75.00%) followed by 2.0 per cent concentration of agniastra (70.00%). The lowest mortality was observed at 5.0 per cent aqueous leaf extract (35.00%) followed by 5.0 per cent diethyl ether leaf extract of eucalyptus (40.00%).

At 48 h exposure period, the highest mortality was attained by 10.0 per cent aqueous leaf extract (83.33%) which was better than all the other extracts and statistically at par with the standard check (86.67%) at 48 h. At 24 h exposure period, larval mortality of 10.0 per cent aqueous leaf extract (66.67%) and 2.0 per cent agniastra (63.33%) was comparable though significantly inferior to standard check. Mean larval mortality increased with increase in exposure period as well as increase in concentration of the extract.

Similar studies by Farsani *et al*., 2011 also concluded that aqueous extracts of tobacco and eucalyptus caused mortality on second instar larvae of *Lycoriella auripila* and suggested that tobacco and eucalyptus could be considered as potential organic insecticides for the control of sciarid flies in mushroom cultivation farm.

Table 7. Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on larval mortality of *Sciara* sp. at different exposure periods

Treatment	Conc. (%)	Per cent larval mortality at different exposure period		Mean
		24 h	48 h	
Aqueous leaf extract of *E. globulus*	5.0	30.00 (32.99)	40.00 (39.13)	35.00 (36.06)
Aqueous leaf extract of *E. globulus*	7.0	40.00 (39.22)	53.33 (46.90)	46.67 (43.06)
Aqueous leaf extract of *E. globulus*	10.0	66.67 (54.76)	83.33 (66.12)	75.00 (60.44)
Diethyl ether leaf extract of *E. globulus*	8.0	26.67 (30.98)	53.33 (46.90)	40.00 (38.94)
Diethyl ether leaf extract of *E. globulus*	9.0	36.67 (37.21)	60.00 (50.75)	48.33 (43.98)
Diethyl ether leaf extract of *E. globulus*	10.0	50.00 (44.98)	73.33 (58.98)	61.67 (51.98)
Agniastra	2.0	63.33 (52.75)	76.67 (61.19)	70.00 (56.97)
B. thuringiensis	0.0001	33.33 (35.20)	53.33 (46.90)	43.33 (41.05)
B. thuringiensis	0.0005	46.67 (43.06)	63.33 (52.75)	55.00 (47.91)
Nuvan	0.1	80.00 (63.41)	86.67 (68.83)	83.33 (66.12)
Untreated control		0	0	0
Mean		43.03 (39.51)	58.48 (48.95)	
$CD_{0.05}$				
	Treatment (T)	6.43		
	Exposure Period (P)	2.74		
	T×P	9.09		

Figures in the parentheses are angular transformed values

Shamshad *et al*., 2008 tested *B. thuringiensis* against *Lycoriella ingenua* and found it to be ineffective against second and third instar larvae when applied as a drench treatments in the laboratory. They concluded that early instar larvae were more susceptible to *B. thuringiensis* than the later instars.

4.3.3 Evaluation of LC_{50} and LC_{90} `for different extracts of *E. globulus* against dipteran flies

Different concentrations of aqueous/ ethanolic/ diethyl ether extracts of *E. globulus* were tested for 2nd and 3rd instar larvae and LC_{50} and LC_{90} were calculated using Probit analysis for *B. asiatica* and *Sciara* sp.

From the data presented in Table 8 it is evident that LC_{50} and LC_{90} values were lower at 48 h when compared to 24 h exposure period. The aqueous leaf extract of eucalyptus gave LC_{50} and LC_{90} values of 6.92 and 14.12 per cent, respectively for *B. asiatica* at 24 h exposure period. While at 48 h it was reduced to 5.37 and 10.71 per cent, respectively. For the same species, LC_{50} and LC_{90} at 24 h were 9.12 and 12.59 per cent followed by 8.50 and 12.88 per cent at 48 h for diethyl ether leaf extract of eucalyptus. For *Sciara* sp., LC_{50} and LC_{90} for aqueous leaf extract were 7.58 and 19.05 per cent, respectively at 24 h exposure period while at 48 h it was 5.89 and 12.88 per cent. For the same species, LC_{50} and LC_{90} evaluated for diethyl ether extract were 10.00 and 15.84 per cent at 24 h followed by 7.76 and 13.29 per cent, respectively.

Farsani *et al*., 2011 also calculated LC_{10} and LC_{50} for aqueous extracts of eucalyptus and tobacco against second instar larvae of *Lycoriella auripila* and evaluated LC_{50} to be 6.49, 0.68 and 0.33 per cent for eucalyptus after 24, 48 and 72 h, respectively.

Table 8. Evaluation of LC_{50} and LC_{90} for different extracts of *E. globulus* against dipteran flies

Treatment	Dipteran fly	At 24 h		At 48 h	
		LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)
Aqueous extract of *E. globulus*	*Bradysia asiatica*	6.92	14.12	5.37	10.71
Diethyl ether extract of *E. globulus*	*Bradysia asiatica*	9.12	12.59	8.50	12.88
Aqueous extract of *E. globulus*	*Sciara* sp.	7.58	19.05	5.89	12.88
Diethyl ether extract of *E. globulus*	*Sciara* sp.	10.00	15.84	7.76	13.49

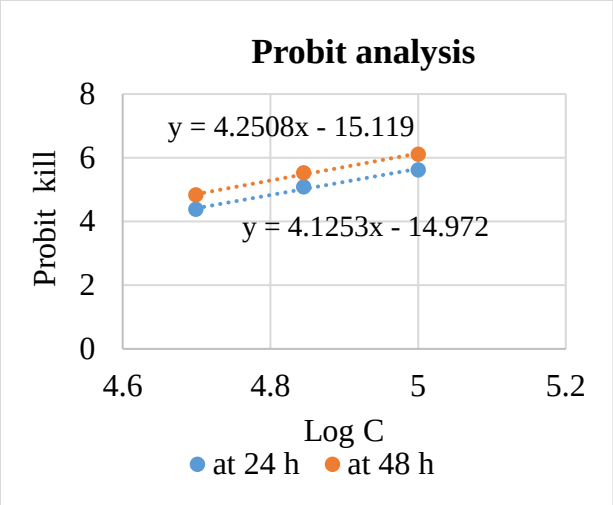


Figure 1 Probit analysis for eucalyptus aqueous leaf extract against *B. asiatica*

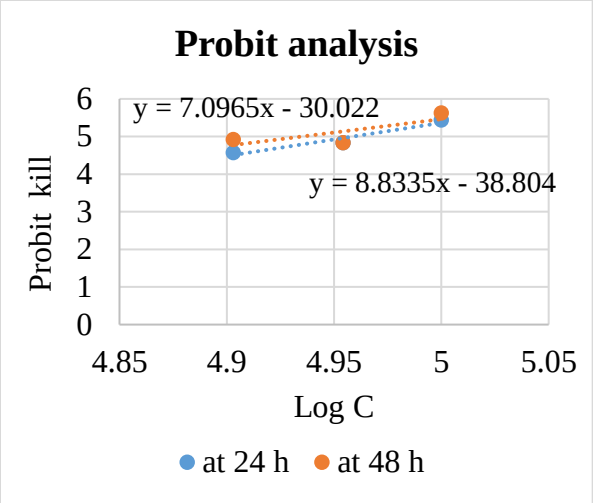


Figure 2 Probit analysis for eucalyptus diethyl ether leaf extract against *B. asiatica*

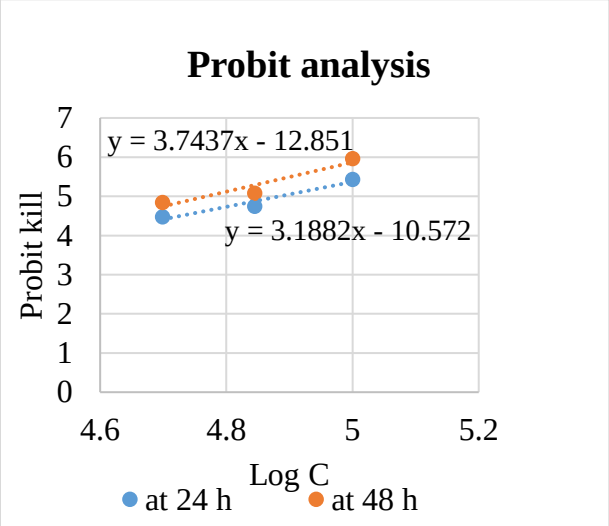


Figure 3 Probit analysis for eucalyptus aqueous leaf extract against *Sciara* sp.

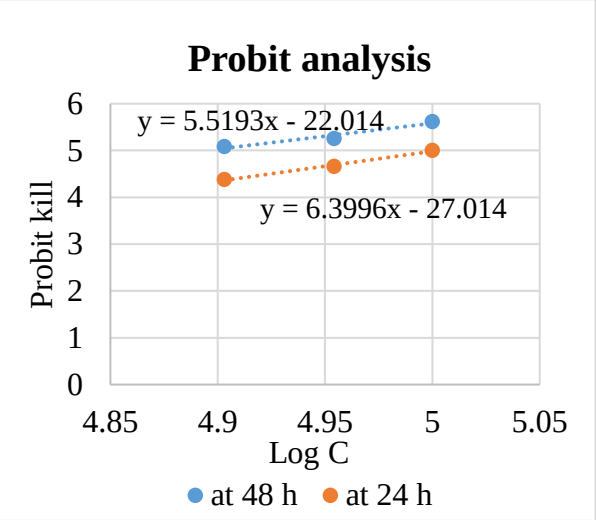


Figure 4 Probit analysis for eucalyptus diethyl ether leaf extract against *Sciara* sp.

4.4 Efficacy of different coloured sticky traps against dipteran flies associated with the oyster mushroom (*Pleurotus* spp.)

Double sided different coloured sticky traps (10×20 cm) *viz.* yellow, blue, green, red and white with bulbs (0.5 Watt) and without bulbs were used (Plate 10). The traps were divided into squares and coated with a layer of mustard oil.

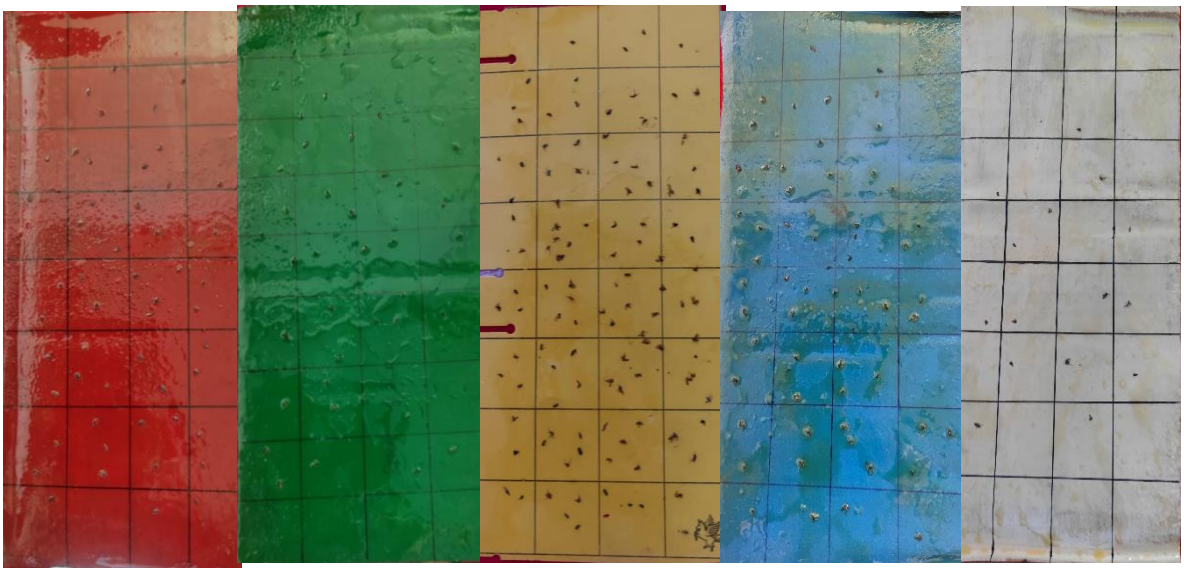


Plate 10 Different coloured sticky traps against *Bradysia asiatica*

Plate 11 Repellency effect on *Bradysia asiatica*

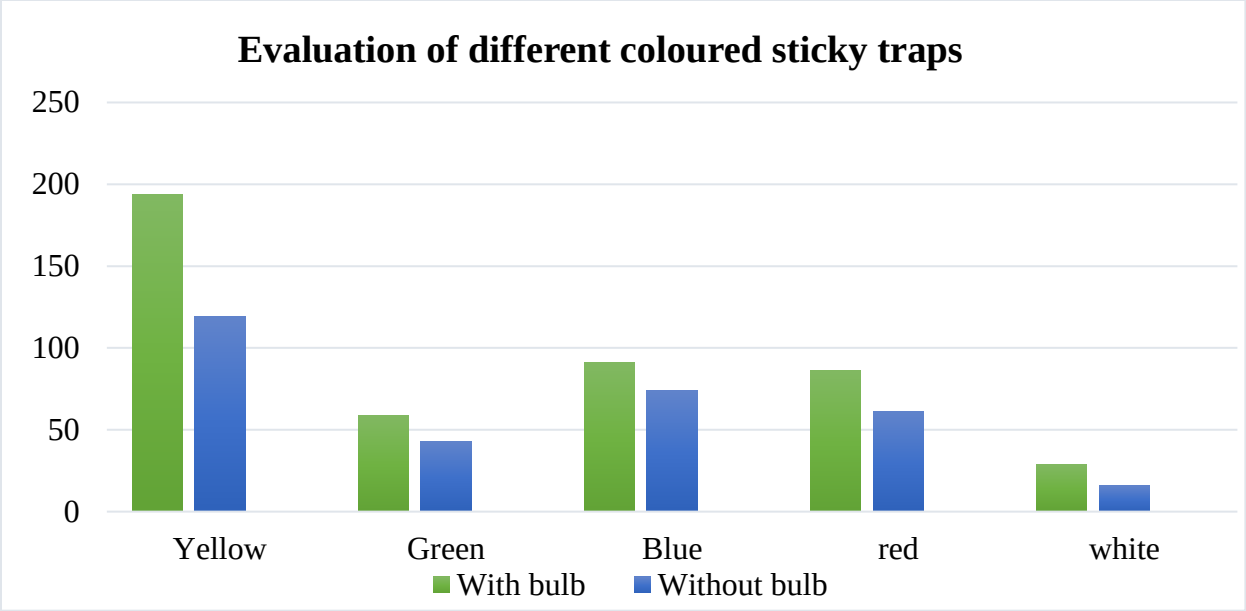


Figure 5 Effect of different coloured sticky traps against *B. asiatica*

Mushroom bags infested with flies were kept in a room for a period of about 10 days. The traps were randomly hung at different locations on the walls of the room. The efficacy of coloured sticky traps was evaluated by counting the number of flies that were stuck to the trap after 10 days. Color of the traps preferred by the pest and number of flies sticking to each trap is presented in Table 9.

Table 9. Efficacy of different coloured sticky traps against *B. asiatica*

Traps	**With Bulb**	**Without bulb**	**Mean**
Yellow	194.00	119.00	156.50
Green	59.00	43.00	51.00
Blue	91.00	74.00	82.50
Red	86.00	61.00	73.50
White	29.00	16.00	22.50
Mean	91.80	62.60	
	Trap (T)	39.67	
	Bulb (B)	25.08	
	T×B	-	

The results revealed significantly higher number of flies sticking to the traps with bulb (91.80) in comparison to the traps without bulb (62.60). Yellow was the most preferred color as maximum flies were found sticking to it (156.50). Not much difference was observed between blue (82.50) and red traps (73.50) while white traps were the least effective and caught minimum number of flies.

Sahin *et al*., 2016 performed a similar experiment to elucidate the effectiveness of yellow and blue coloured sticky traps in capturing the mushroom flies. Manzin *et al*., 2019 also studied the activity and population of mushroom phorid fly, *Megaselia halterata* using yellow sticky traps.

4.5 Repellent effect of leaf extracts of *E. globulus* on *B. asiatica*

Repellent effect of different eucalyptus formulations was studied under laboratory conditions. 100 ml beakers containing compost treated with 10.0 per cent aqueous/ diethyl ether/ ethanol were kept in a cage (Plate 11). A control without any treatment was also kept along with it.

Table 10. Repellent effect of leaf extracts of *E. globulus* on adults of *B. asiatica*

Treatment	Per cent repellency
Aqueous leaf extract of *Eucalyptus* spp.	25.00 ± 7.22
Ethanolic leaf extract of *Eucalyptus* spp.	54.17 ± 4.17
Diethyl ether leaf extract of *Eucalyptus* spp.	70.83 ± 4.17
Untreated control	0
C.D. SE(m) SE(d) C.V.	15.43 4.66 6.59 21.52

Twenty adults of *B. asiatica* were introduced into the cage and after 24 h repellency was checked by observing the number of flies that landed on the treated beakers and control. The same experiment was replicated thrice. Repellency rate is presented in the Table 10.

The best repellency rate was shown by eucalyptus diethyl ether leaf extract (70.83%) followed by ethanolic extract (54.17%). Aqueous leaf extract of eucalyptus was found to be least effective (25.00%) for repellency against mushroom flies. Akob and Ewete, 2009 performed a

similar experiment and tested ethanolic extracts of *E. grandis* along with 3 other plants against *Sitophilus zeamais* and found varying percentage of repellency by all four plants.

Chapter-5

SUMMARY AND CONCLUSIONS

The present investigation entitled **"Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)"** was carried out in the laboratory conditions of COHF, Neri. Incidence of flies on the oyster mushroom was recorded, biology of two major flies was studied and their management was carried out using aqueous/ diethyl ether/ ethanolic extracts of *Eucalyptus globulus*, angiastra and *Bacillus thuringiensis*. Repellent property of eucalyptus extracts against adult flies was also evaluated. Preference of trap colour by the adult flies was learned. The results obtained from the present studies are briefly summarized and concluded here under:

5.1 Incidence of dipteran flies on oyster mushroom (*Pleurotus* spp.)

Incidence of the pest recorded from the districts of Hamirpur, Kullu and Kangra indicate that the highest fly infestation was found in the district of Hamirpur (62.86%) followed by Kullu (51.33%) and Kangra (42.7%). The mean pest incidence was 52.55 per cent for the three districts. The larvae were seen damaging the sporophores and compost mycelium. Sciarid flies were more prevalent than phorid flies in the state. They caused great yield loss since the damaged mushrooms could not be marketed or consumed.

5.2 Identification of dipteran flies from zoological survey of India, Kolkata

The fly samples collected from the various mushroom units surveyed were sent to ZSI, Kolkata for identification and the species identified from Kullu were *Bradysia asiatica Megaselia* sp. and *Sciara* sp. while from Hamirpur, *Sciara orientalis* was identified.

5.3 Biology of *Bradysia asiatica* on oyster mushroom (*Pleurotus* spp.)

The life cycle of *B. asiatica* studied under laboratory conditions revealed fecundity rate of 21.67 ± 2.03 eggs for the female fly was. The pre-oviposition period and oviposition period was completed in 1.67 ± 0.33 and 3 ± 0.58 days respectively while the incubation period was completed in 3.33 ± 0.33 days. The eggs were oval and translucent and the larvae were

apodous, slender with a well sclerotized head capsule. Larvae went through 4 instar development and the period was completed in a lapse of 10.67 ± 0.33 days. Newly formed pupae were white which later turned yellow and finally into golden brown. The pupal period and adult longevity was 4.33 ± 0.33 and 4.67 ± 0.33 days, respectively and the total life cycle was completed in 23 ± 1 days.

5.4 Biology of *Sciara* sp. on oyster mushroom (*Pleurotus* spp.)

The fecundity rate for *Sciara* sp. was 28.67 ± 2.03 eggs. Pre- oviposition and oviposition period lasted for 1.33 ± 0.33 and 3.33 ± 0.33 days respectively. The eggs of the fly were of dirty white colour which turned transparent before hatching. The incubation period was of 2.33 ± 0.33 days while the larval development took about 10.67 ± 0.33 days and the larvae moulted at least three times to turn into a pupa. The fourth instar larva became sluggish, stopped feeding and spun a cocoon for pupation. The pupa resembled adult fly and had appendages close to the body. It took 4.33 ± 0.33 days for the pupa to turn into an adult fly. The adult longevity was 4 ± 0.58 days and the total life cycle was completed in 21.33 ± 0.67 days.

5.5 Evaluation of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on the growth of mushroom mycelia of *Pleurotus* spp.

Out of the twelve treatments and a control for evaluating mycelial inhibition using leaf extracts of eucalyptus *viz.* aqueous (5.0, 7.0, 10.0%)**,** ethanolic (7.0, 9.0, 10.0%), diethyl ether (8.0, 9.0, 10.0 %), agniastra (2.0%) and *B. thuringiensis* (0.0001 and 0.0005 %) prepared through poison food technique, it was found that there was complete mycelial inhibition (100.00%) for ethanolic extracts of *E. globulus*. The inhibitory effect of *B. thuringiensis* (0.0001%) was 34.17 per cent while 10.0 per cent diethyl ether extract gave 21.90 per cent inhibition. No mycelial inhibition was shown by agniastra (2.0%), aqueous extracts (5.0, 7.0, 10.0%) and 8.0 per cent diethyl ether extract of *E. globulus*.

5.6 Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on larval mortality of *B. asiatica* at different exposure periods

Larval mortality was estimated by using 2nd and 3rd instars of *B. asiatica* for various eucalyptus leaf extract concentrations, *B. thuringiensis* and agniastra in comparison to a standard (nuvan @ 0.1%). In case of *B. asiatica,* highest larval mortality was achieved at 10.0 per cent aqueous leaf extract of eucalyptus (80.00%) followed by 2.0 per cent agniastra (75.00%). The lowest rate of mortality was shown by 5.0 per cent aqueous leaf extract and 0.0001 per cent *B. thuringiensis* (35%). There was a progressive increase in mortality with increase in exposure period as well as increase in the concentration of extracts. The mean larval mortality was significantly higher at an exposure period of 48 h (59.69%) in comparison to 24 h (46.36%). At 48 h exposure period maximum mortality was shown by 10.0 per cent aqueous extract (86.67%) followed by 2.0 per cent agniastra (83.33%) and 10.0 per cent diethyl ether leaf extract (80.00%).

5.7 Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on larval mortality of *Sciara* sp. at different exposure periods

When the extracts were tested against larvae of *Sciara* sp., almost similar results were obtained. The highest mortality was attained at 10.0 per cent concentration of aqueous leaf extract (75.00%) followed by 2.0 per cent concentration of agniastra (70.00%). The lowest mortality was at 5.0 per cent aqueous leaf extract (35.00%) followed by 5.0 per cent diethyl ether leaf extract (40.00%). The maximum mean mortality was achieved at 48 h exposure period (58.48%) followed by 24 h (43.03%). At 48 h exposure period, the highest mortality was given by 10.0 per cent aqueous leaf extract (83.33%) which was better than all other extracts in relation to the standard (86.67%) at 48 h.

5.8 Evaluation of LC_{50} and LC_{90} for different extracts of *E. globulus* against dipteran flies

LC_{50} and LC_{90} estimated for *B. asiatic*a at 24 h were 6.92 and 14.12 per cent and at 48 h were 5.37 and 10.71 per cent for aqueous leaf extract of eucalyptus while it was 9.12 and 12.59 per cent at 24 h, and 8.5 and 12.88 per cent at 48 h for diethyl ether leaf extract of eucalyptus. For *Sciara* sp., it was 7.58 and 19.05 per cent at 24 h and 5.89 and 12.88 per cent at 48 h for

aqueous leaf extract of eucalyptus while it was 10.00 and 15.84 per cent at 24 h and, 7.76 and 13.49 per cent at 48 h for diethyl ether leaf extract of eucalyptus.

5.9 Efficacy of different coloured sticky traps against *B. asiatica*

Double sided coloured sticky traps (10×20 cm) *viz.* yellow, blue, green, red and white with bulbs (0.5 Watt) and without bulbs were used. Significantly greater number of flies were found sticking to the traps with bulbs (91.80) than without bulbs (62.60). Yellow traps were the most effective with 156.50 flies trapped followed by red, blue and green traps. While white traps (22.50) were the least efficient in control of flies.

5.10 Repellent effect of leaf extracts of *E. globulus* on adults of *B. asiatica*

Repellency effect of different eucalyptus formulations at 10.0 per cent concentration was tested against the adults of *B. asiatica*. The best repellency rate was shown by eucalyptus diethyl ether leaf extract (70.83%) followed by ethanolic extract (54.17%). Aqueous leaf extract of eucalyptus was found to be least effective (25.00%) for repellency against mushroom flies.

From the present study, we can conclude that the mushroom fly activity was higher in Hamirpur as compared to Kangra and Kullu districts. The biology of both the species indicate that it can complete more than one generation on a single crop. Therefore, its severity can be increased many fold as its reproductive potential is very high. Their development was fast at high temperature. Since mushroom is a short duration crop and use of insecticides will lead to residual effects. Therefore, botanicals were tested for the control of the pest. 10.0 per cent aqueous leaf extract of eucalyptus caused larval mortality of 80.00 and 75.00 per cent followed by 2.0 per cent agniastra (75.00% and 70.00%) for *B. asiatica* and *Sciara* sp., respectively. Diethyl ether leaf extract of eucalyptus provides good repellency rate of 70.83 per cent followed by ethanolic leaf extract of eucalyptus (54.17%). The biopesticides are safer to the environment with no residual effect. Yellow sticky traps with and without bulbs play an important role in providing maximum fly catch followed by blue and red coloured traps.

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APPENDIX – I

ANOVA TABLES

Analysis of Variance (ANOVA) Table 3: Biology of *Bradysia asiatica* on oyster mushroom (*Pleurotus* spp.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	6	1,018.48	169.746	209.686	0
Error	14	11.333	0.81		
Total	20	1,029.81			

Analysis of Variance (ANOVA) Table 4: Biology of *Sciara* sp. on oyster mushroom (*Pleurotus* spp.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	6	905.81	150.968	264.194	0
Error	14	8	0.571		
Total	20	913.81			

Analysis of Variance (ANOVA) 5: Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on the growth of mushroom mycelia of *Pleurotus* spp.

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	12	510.446	42.537	13,644.75	0
Error	26	0.081	0.003		
Total	38	510.527			

Analysis of Variance (ANOVA) Table 6: Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on larval mortality of *Bradysia asiatica* at different exposure periods

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment (T)	10	21,499.59	2,149.96	117.753	0
Exposure period (P)	1	1,171.52	1,171.52	64.164	0
Intraction T X P	10	223.634	22.363	1.225	0.3023
Error	44	803.359	18.258		
Total	65	23,698.10			

Analysis of Variance (ANOVA) Table 7: Effect of leaf extracts of *E. globulus*, agniastra and *B. thuringiensis* on larval mortality of *Sciara* sp. at different exposure periods

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment (T)	10	18,242.22	1,824.22	149.922	0
Exposure period (P)	1	1,472.08	1,472.08	120.982	0
Intraction T X P	10	312.996	31.3	2.572	0.01517
Error	44	535.382	12.168		
Total	65	20,562.67			

Analysis of Variance (ANOVA) Table 8: Evaluation of LC_{50} and LC_{90} for different extracts of *E. globulus* against dipteran flies

Set 1: Probit analysis for *Bradysia asiatica* at 24 h with eucalyptus aqueous leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.771300982	0.771300982	113.6137817	0.059551851
Residual	1	0.006788798	0.006788798		
Total	2	0.77808978			

Set 2: Probit analysis for *Bradysia asiatica* at 48 h with eucalyptus aqueous leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.818939858	0.818939858	241.9213682	0.040873863
Residual	1	0.003385149	0.003385149		
Total	2	0.822325007			

Set 3: Probit analysis for *Bradysia asiatica* at 24 h with eucalyptus diethyl ether leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.366793	0.366793	14.82771	0.161753626
Residual	1	0.024737	0.024737		
Total	2	0.39153			

Set 4: Probit analysis for *Bradysia asiatica* at 48 h with eucalyptus diethyl ether leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.236727	0.236727	1.695552	0.41692434
Residual	1	0.139616	0.139616		
Total	2	0.376343			

Set 5: Probit analysis for *Sciara* sp. at 24 h with eucalyptus aqueous leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.460688989	0.460688989	18.56313194	0.145188507
Residual	1	0.024817417	0.024817417		
Total	2	0.485506407			

Set 6: Probit analysis for *Sciara* sp. at 48 h with eucalyptus aqueous leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.635193777	0.635193777	10.14766061	0.193644918
Residual	1	0.062595095	0.062595095		
Total	2	0.697788872			

Set 7: Probit analysis for *Sciara* sp. at 24 h with eucalyptus diethyl ether leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.143191444	0.143191444	16.52800908	0.153543899
Residual	1	0.008663563	0.008663563		
Total	2	0.151855007			

Set 8: Probit analysis for *Sciara sp.* at 48 h with eucalyptus diethyl ether leaf extract

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Regression	1	0.192515609	0.192515609	135.6625227	0.054523845
Residual	1	0.001419077	0.001419077		
Total	2	0.193934687			

Analysis of Variance (ANOVA) Table 9: Efficacy of different coloured sticky traps against *Bradysia asiatica*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Traps (T)	4	60,052.80	15,013.20	14.035	0.00001
Bulbs (B)	1	6,394.80	6,394.80	5.978	0.02387
Intraction TXB	4	4,051.20	1,012.80	0.947	0.45763
Error	20	21,394.00	1,069.70		
Total	29	91,892.80			

Analysis of Variance (ANOVA) Table 10: Repellent effect of leaf extracts of *E. globulus* on adults of *Bradysia asiatica*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	8,854.17	2,951.39	45.333	0.00002
Error	8	520.833	65.104		
Total	11	9,375.00			

APPENDIX – II

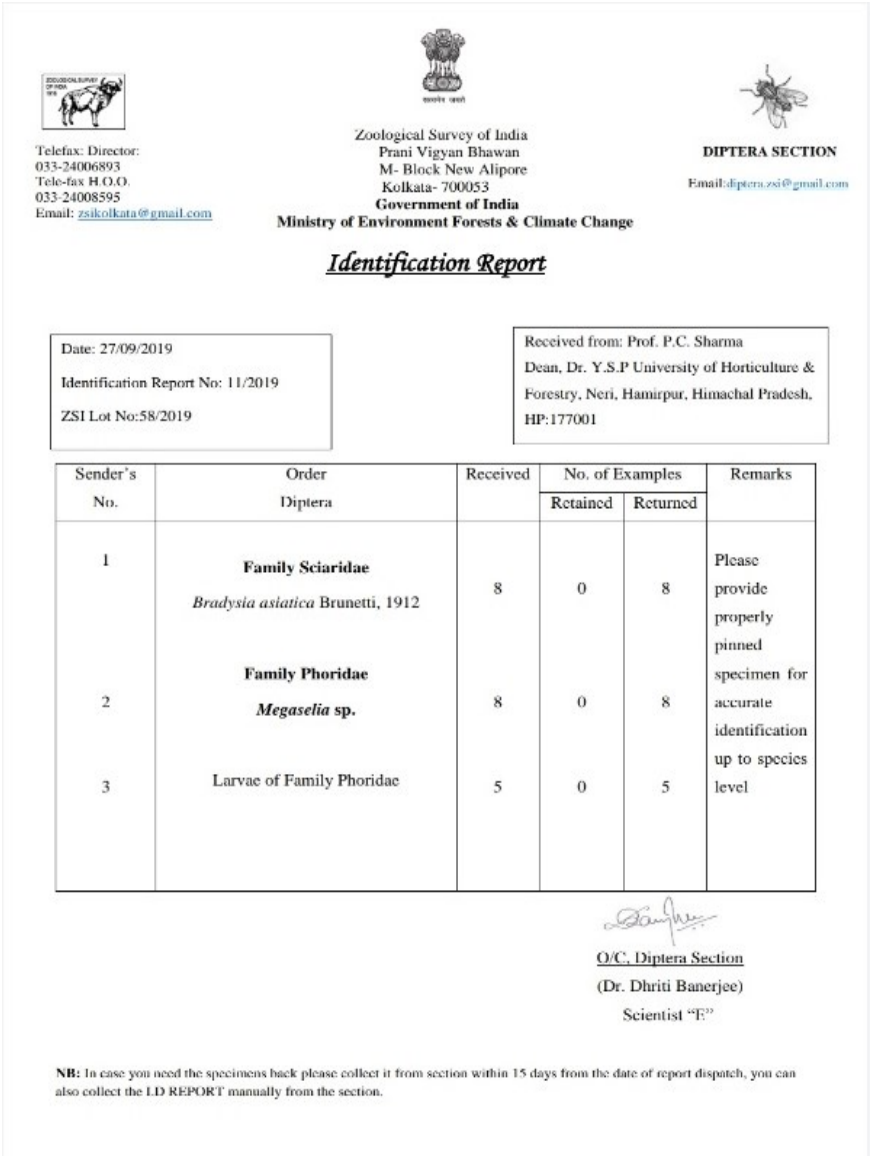
COMPOSITION OF MEDIA

S. No.	Ingredients	Composition
1.	Malt extract	25.0 g
2.	Agar- agar	20.0 g
3.	Distilled water	1000.0 ml

APPENDIX – III

IDENTIFICATION REPORTS

Bradysia asiatica and *Megaselia* sp. (Vial 1) collected from Kullu district.



Telefax: Director:
033-24006893
Tele-fax H.O.O.
033-24008595
Email: zsikolkata@gmail.com

Zoological Survey of India
Prani Vigyan Bhawan
M- Block New Alipore
Kolkata- 700053
Government of India
Ministry of Environment Forests & Climate Change

DIPTERA SECTION
Email:diptera.zsi@gmail.com

Identification Report

Date: 27/09/2019
Identification Report No: 11/2019
ZSI Lot No:58/2019

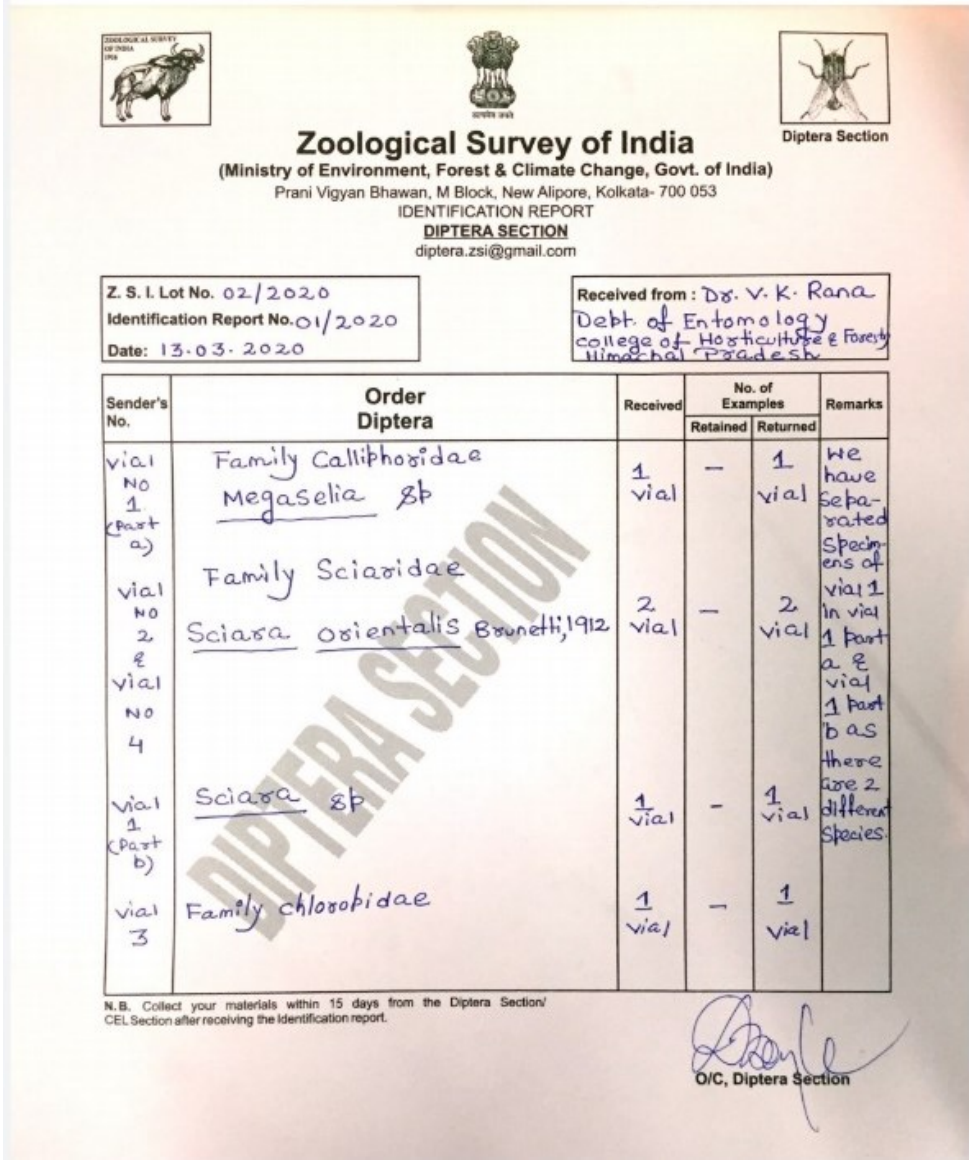
Received from: Prof. P.C. Sharma
Dean, Dr. Y.S.P University of Horticulture & Forestry, Neri, Hamirpur, Himachal Pradesh, HP:177001

Sender's No.	Order Diptera	Received	No. of Examples		Remarks
			Retained	Returned	
1	**Family Sciaridae** *Bradysia asiatica* Brunetti, 1912	8	0	8	Please provide properly pinned specimen for accurate identification up to species level
2	**Family Phoridae** ***Megaselia* sp.**	8	0	8	
3	Larvae of Family Phoridae	5	0	5	

O/C, Diptera Section
(Dr. Dhriti Banerjee)
Scientist "E"

NB: In case you need the specimens back please collect it from section within 15 days from the date of report dispatch, you can also collect the I.D REPORT manually from the section.

Sciara orientalis (Vial 2) collected from Hamirpur district.



Zoological Survey of India

(Ministry of Environment, Forest & Climate Change, Govt. of India)

Prani Vigyan Bhawan, M Block, New Alipore, Kolkata- 700 053

IDENTIFICATION REPORT

DIPTERA SECTION

diptera.zsi@gmail.com

Diptera Section

Z. S. I. Lot No. 02/2020

Identification Report No. 01/2020

Date: 13.03.2020

Received from : Dr. V. K. Rana
Dept. of Entomology
college of Horticulture & Forestry
Himachal Pradesh

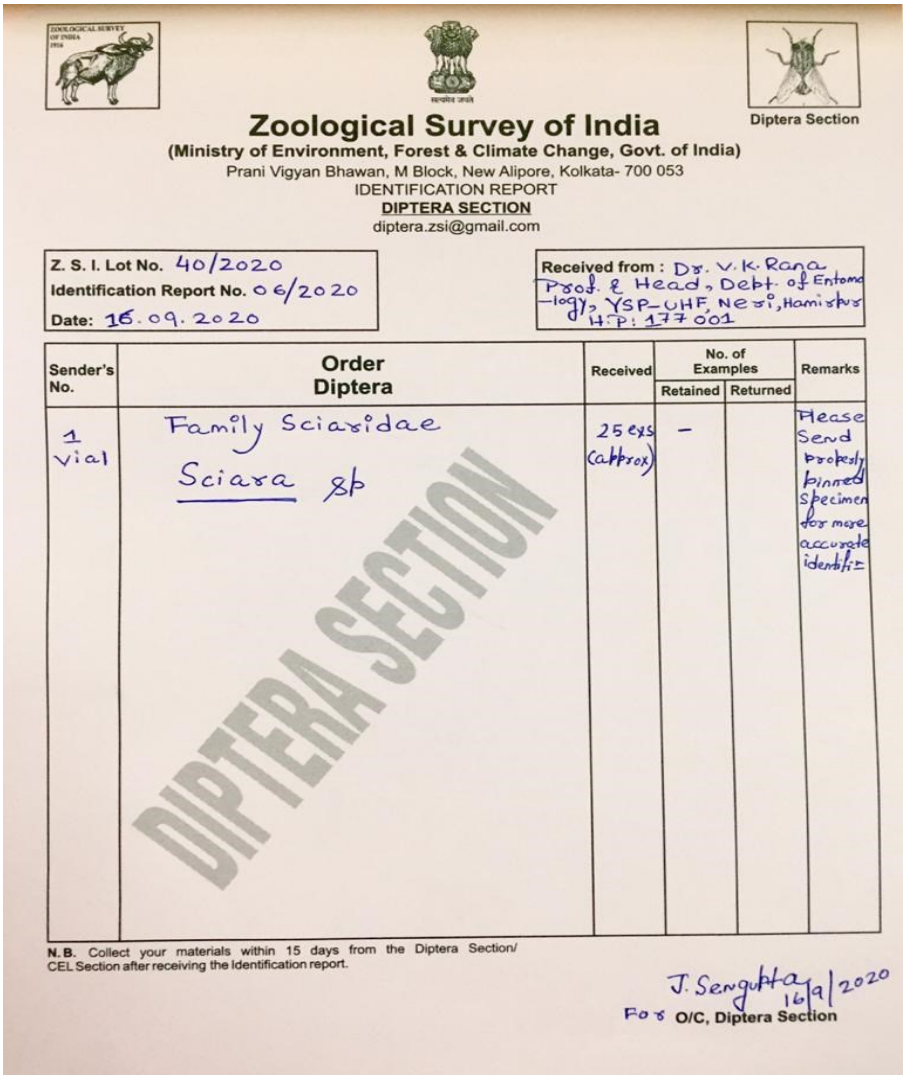
Sender's No.	Order Diptera	Received	No. of Examples Retained	No. of Examples Returned	Remarks
vial No 1 (Part a)	Family Calliphoridae Megaselia sp	1 vial	–	1 vial	We have Separated Specimens of vial 1 in vial 1 Part a & vial 1 Part b as there are 2 different Species.
vial No 2 & vial No 4	Family Sciaridae Sciara orientalis Brunetti, 1912	2 vial	–	2 vial	
vial 1 (Part b)	Sciara sp	1 vial	–	1 vial	
vial 3	Family chloropidae	1 vial	–	1 vial	

DIPTERA SECTION

N.B. Collect your materials within 15 days from the Diptera Section/ CEL Section after receiving the Identification report.

O/C, Diptera Section

Sciara sp. (Vial 1) collected from Kullu district



Zoological Survey of India

(Ministry of Environment, Forest & Climate Change, Govt. of India)

Prani Vigyan Bhawan, M Block, New Alipore, Kolkata- 700 053

IDENTIFICATION REPORT

DIPTERA SECTION

diptera.zsi@gmail.com

Diptera Section

Z. S. I. Lot No. 40/2020

Identification Report No. 06/2020

Date: 16.09.2020

Received from : Dr. V. K. Rana Prof. & Head, Dept. of Entomology, YSP-UHF, Nesi, Hamirpur HP: 177 001

Sender's No.	Order Diptera	Received	No. of Examples		Remarks
			Retained	Returned	
1 vial	Family Sciaridae Sciara sp	25 exs (approx)	–		Please Send properly pinned specimen for more accurate identification

DIPTERA SECTION

N.B. Collect your materials within 15 days from the Diptera Section/ CEL Section after receiving the Identification report.

J. Sengupta 16/9/2020

For O/C, Diptera Section

Department of Entomology
Dr. Y. S. Parmar University of Horticulture and Forestry,
Nauni, Solan-173230 (H.P)

Title of Thesis	**:**	**Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)**
Name of the Student	**:**	**Devika Sharma**
Admission Number	**:**	NH-2018-04-M
Major Advisor	**:**	Dr. Sunil Kumar
Major Field	**:**	Entomology
Minor Field	**:**	Plant Pathology
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ABSTRACT

The present investigation entitled "Incidence, biology and bio-intensive management of dipteran flies in oyster mushroom (*Pleurotus* spp.)" was carried out to investigate the incidence of mushroom flies in three districts of Himachal Pradesh *viz*., Hamirpur, Kullu and Kangra. The life cycle of *Bradysia asiatica* and *Sciara* sp. was studied which were completed in 23 days and 21 days, respectively. The bio-efficacy studies of different leaf extracts of eucalyptus against *B. asiatica* and *Sciara* sp. encountered during the survey indicated that overall per cent larval mortality at 10% aqueous leaf extract of eucalyptus were 80 and 75 while for agniastra it was 75 and 70 per cent, respectively. Minimum larval mortality was attained at 5% eucalyptus aqueous leaf extracts and 0.0001 per cent *B. thuringiensis*. The maximum per cent repellent activities were shown by eucalyptus diethyl ether extract (70.83%) followed by ethanolic extract (54.17%) against the adults of *B. asiatica*. In another expirement, yellow coloured sticky trap with and without bulb were the most efficient in attracting maximum number of flies while the white coloured trap attracted least no of adult flies. From the present investigation, we can conclude that dipteran flies had short life cycle and were prevalent throughout the state. Keeping in view the toxicity of synthetic insecticides, indigenous plant material having insecticidal properties can be used along with non-chemical methods to manage these flies in *Pleurotus* spp.

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