STUDIES ON INCIDENCE, PATHOGENICITY, BIOLOGY AND BIOMANAGEMENT OF INSECT PESTS ASSOCIATED WITH CULTIVATED MUSHROOMS

Thesis

by

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Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

ENTOMOLOGY AND APICULTURE

COLLEGE OF HORTICULTURE

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2010

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CERTIFICATE-I

This is to certify that the thesis entitled, **"Studies on incidence, pathogenicity, biology and biomanagement of insect pests associated with cultivated mushrooms",** submitted in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **ENTOMOLOGY AND APICULTURE** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a record of bonafide research work carried out by **Mr. Anurag Sharma (H-2006-04-D)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

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Chairperson
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Dated: 14th May, 2010
Place: Nauni, Solan

CERTIFICATE-II

This is to certify that the thesis entitled, **"Studies on incidence, pathogenicity, biology and biomanagement of insect pests associated with cultivated mushrooms",** submitted by **Mr. Anurag Sharma(H-2006-04-D)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **ENTOMOLOGY AND APICULTURE** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external 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, **"Studies on incidence, pathogenicity, biology and biomanagement of insect pests associated with cultivated mushrooms"** submitted to Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) by **Mr. Anurag Sharma (H-2006-04-D)** in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **ENTOMOLOGY AND APICULTURE.**

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Acknowledgements

*With immense humility, I bow in devotion to **'Lord Siva'** for bestowing me with physical, mental and spiritual strength to shoulder the responsibilities of life.*

Words are beyond the lexicon to express my gratitude to my reverend parents Smt. Sukanya Sharma and Sh. R. K. Sharma whose dedication and inspiration kept me off the worries and responsibilities of my share during tenure of these studies. Minus their supreme sacrifice, this treatise *would have remained an imagination. It is the fruition of their patience, encouragement and blessings that I could climb the ladder of education step by step to reach this level.*

It gives me immense pleasure to express my highest venerations and heart felt gratitude *to my esteemed and dignified teacher Dr (Mrs.) Anju Sudhakar Khanna, chairperson of my advisory committee for* her innovative ideas, keen interest, judicious, impeccable and benevolent guidance, concrete suggestions, constant inspiration and sincere help during the course of study and preparation of this manuscript. *I shall remain ever indebted for the maternal love she conferred upon me.*

I emphatically extend my gracious thanks to Dr. Divender Gupta, Dr. B. C Suman and Dr. (Mrs.) Nivedita Sharma, the worthy members of my advisory committee for their counsel, supportive attitude and interest in scrutinizing this manuscript.

The help rendered by Dr. P. R. Gupta, Professor and Head, Department of Entomology and Apiculture, during course of this study is gratefully acknowledged.

I place on record my revered thanks to Dr. V. V. Ramamurthy, Professor and Head, Department of Entomology, IARI, New Delhi for identifying the insect pests encountered in the cropping bags of cultivated edible mushrooms during present course of investigation .

The love and affection showered by my beloved sister, Shikha is beyond the words of acknowledgement.

It is with personal touch of emotions that I cherish the soothing care, inveterate affection, moral support and love of Reecha, my fiancee, who has influenced my life in many positive ways.

It is a pleasure to express my feelings for Sudhakar Sir, Suvidha and Sahiba for providing family atmosphere during my visits to their residence.

A word of special thanks awaits for *the office and laboratory staff of Department of Entomology & Apiculture and Department of Horticulture, Solan (HP).*

The help and co-operation rendered by the field staff of University Mushroom Farms at Chambaghat and Nauni is greatfully acknowledged.

I heartily express my thanks to respected senior and friends Dr. Jariyal, Pooja, Manju, Rajeev and Ritesh for their moral support that made my stay at Solan a reminiscence.

Last but not the least, I am thankful for direct and indirect help received from various quarters but not mentioned here because of slip of mind and pen.

Nauni, Solan

Dated : 14st May, 2010 (Anurag Sharma)

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CHAPTER-1

INTRODUCTION

Indian agriculture essentially needs to continuously evolve so that it remains ever responsive to meet the growing and diversified needs of different stakeholders in the entire production to consumption chain. Mushroom production represents one of the most significant commercial steps towards diversification of agriculture based on microbial technology for large-scale recycling of agro-wastes and their transformation into edible biomass, accepted as highly nutritive food with royal flavor and palatability. It relieves the pressure on arable land as its cultivation is indoors, and so, is more suitable for the women folk. Being an agriculture base country, India offers immense potential for the cultivation of mushrooms as agro waste that forms the main ingredient of substrate medium for mushroom growing is available in plenty.

Cultivated mushrooms are the delicious edible fungi with distinct fruit bodies that supplement and complement the nutritional deficiencies of vitamins and minerals and are regarded as the highest producers of high quality digestive proteins per unit area and time. They comprise a healthy food virtually free from fat and cholesterol (Shivanna *et al*., 2003). Their excellent culinary properties make them the favorite recipe of the kitchens world over. It is for this reason that the total global mushroom production is increasing manifold year after year (Dhar, 1997).

The important commercially cultivated mushrooms in the nation are, white button mushroom, *Agaricus bisporus* (Lange) Imbach, oyster mushroom, *Pleurotus* spp. and paddy straw mushroom, *Volvariella* spp. (Tewari, 2005). Of these, major share is of *A. bisporus* with 85.0 per cent of the total of 70,000 tones of annual production in the country coming out of this mushroom. Its wide acceptability is due to its majestic buttons, essence and numerous pleasantly tasting recipes (Lamba *et al*., 2007). Even in global scenario, one third share of the total mushroom production goes to this species (Chang, 1996). Oyster mushroom (*Pleurotus* spp.) stands second only to button mushroom in production, contributing 24.1 per cent of the total world commercial mushroom production due to its desirable attributes like unique flavor, exotic taste, rich in protein, vitamins and minerals, and more importantly the rapid mycelial growth, high ability to directly degrade lignocellulosic farm wastes, simple and cheap cultivation techniques and ample choice of species available for cultivation under different climatic conditions ((Bahl, 1995; Balakrishnan and Nair, 1997; Khare *et al*., 2007; Kumar, 2006). Paddy straw mushroom (*Volvariella* spp.) is grown in the regions having subtropical and tropical climate. The state of Himachal Pradesh ranks first in the country as far as the cultivation of former two mushrooms is concerned.

Despite numerous appropriate reasons for this industry to rise in India like varied agro-climate, large quantities of agro-wastes and sizeable population of the small and marginal farmers interested to grow mushrooms as an additional source of income, the pace of growth is relatively slow. Reason behind is that most of the cultivation in the country is undertaken by marginal farmers under unhygienic conditions in improvised mushroom farms which prove to be the hot beds for multiplication of various pests and pathogens (Khanna and Kumar, 2005). Pests in the form of arthropods, nematodes and mites are a constant threat to the successful commercial production of mushrooms (Sandhu, 1995; Lewandowski *et at.,* 1999; Khanna and Chandran, 2002; Kumar *et al.,* 2007a and Kumar *et al.,* 2007b).

Arthropod pests of concern in mushroom cultivation are dipteran flies, coleopteran beetles and spring tails. Most abundant and menacing among them, are small to medium size delicate dipteran flies often bearing resemblance to gnats and midges. These flies are exceedingly numerous in individuals and species with a wide geographical range (Richards and Davies, 1977; Brown and Marshal, 1984; Chakarvarty *et al.,* 1987; Brar and Sandhu, 1989; Alekseeva and Sokolova, 1996; Marzo, 1998; Kumar and Sharma, 1998; Kumar and Sharma, 2000; Gnaneswaran and Wijayagunasekara,1999; White and Smith, 2000). More than 13 genera belonging to six families of these flies have been found to be associated with mushroom cultivation. These are the major pests of mushroom throughout the world with extremely low economic threshold levels. Uncontrolled populations of these pests can result in substantial losses in yield due to both, direct feeding by maggots and associated disease spread by adult flies. These factors necessitate the use of management tools even at low larval densities.

Despite being the pests of global significance, information regarding their incidence, biology and damaging potential in two commercially cultivated mushrooms viz. *A. bisporus* and *P. sajor caju* under the conditions existing in the state of Himachal Pradesh is scanty and scattered. Also, the prevailing methods of insect pest control in mushroom production undesirably rely upon the use of insecticides which inevitably needs to be discouraged, looking into the uniqueness and cropping pattern of mushrooms and also due to the residue problem they pose in mushroom fruit bodies which have a short life and are normally consumed immediately after harvest. Preliminary experiments on biomanagement of these pests by use of entomophagous nematodes, insect growth regulators and certain plant products have found some success elsewhere (Grewal *et al.* 1992; Rovesti *et al.* 1996; Szynk and Bednarek, 2003; Lamba *et al*., 2007). This piece of research is thus planned with the following objectives:

- ❖ To conduct the faunistic survey and identification of insect-pests associated with the commercially cultivated edible mushrooms, viz. *Agaricus bisporus* and *Pleurotus sajor caju.*
- ❖ To study the seasonal abundance of sciarids and phorids in the selected mushroom farms growing these mushrooms.
- ❖ To study the biology of one of the most prevalent insect pest of these mushrooms.
- ❖ To evaluate damaging potential of the test insect pest.
- ❖ Biomanagement of the test insect in both the test mushrooms.

CHAPTER-2

REVIEW OF LITERATURE

The present literature relates to the research work conducted during the investigations presented in this thesis. Multifarious aspects like faunistic studies of insects associated with two most important cultivated edible mushrooms viz. *Agaricus bisporus* and *Pleurotus sajor-caju*, their seasonal abundance, biology, damaging potential and biomanagement have been broadly covered. Looking into the objectives of the present work, the available literature has been reviewed under the following heads.

2.1 Incidence of insect and Collembola (Springtails) fauna associated with cultivated edible mushrooms

2.2 Biology and behavior of insect pests on mushrooms

2.3 Seasonal abundance of dipteran pests in mushrooms

2.4 Nature of damage, damage potential and economic threshold level of dipteran flies associated with mushrooms

2.5 Biomanagement of insect pests infesting mushrooms

2.1 Incidence of insect and Collembola fauna associated with cultivated edible mushrooms

Insects belonging to different groups occur in abundance in cultivated edible mushrooms. There is no stage of mushroom cultivation that is not infested by one or the other insect pest. The unhygienic conditions of cultivation in most of the farms in India make them highly prone to these pests. Since the present investigations are confined to button mushrooms (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus* spp.), the information on distribution and faunistic incidence of the pests infesting these mushrooms only have been reviewed. Since the insects attacking the mushrooms are numerous and diverse as far as their order, family, genera and species are concerned, the available information on their incidence has been presented in tabulated form.

2.1.1. Incidence of insect pests associated with *Agaricus* spp. and *Pleurotus* spp.

Insect	Mushroom species	References
Order Diptera		
Family Sciaridae		
Bradysia agrestis Sasakawa	-	Sasakawa and Akamatsu, 1978
B. brunnipes	-	Alekseeva and Sokolova, 1996
B. chikuni	-	Yang and Tan, 1995
B. coprophila Linter	-	Hussey, 1969
B. difformis Frey	*A. bisporus and A. blazei.*	White *et al*., 2000; Menzel *et* al., 2003 and Greenslade and Clift, 2004
B. fenestralis	-	Weigel, 1959
B. impatiens Johansen	-	Thomas, 1942
B. lutaria Winn.	*A. bisporus*	White and Smith, 2000
B.ocellaris (Comstock)	*A. bisporus*	Shamshad *et al.*, 2008
*B. paupera** Toum.	*A. bisporus*, *P. ostreatus*	Shandilya *et al.,* 1975; Gnaneswaran and Wijayagunasekhara, 1999
*B. tritici** Coq	*A. bisporus*	Sandhu and Brar, 1980
B. yangi	-	Yang and Tan, 1995
Bradysia spp.*	*P. ostreatus*, *A. bisporus*	Kumar, 2006
Lycoriella agirici Felt (=*L. auripila** (=*L. castanescens*) (Lengersdorf)	-	Clift, 1981
	Pleurotus sp.	Chakravarty *et al.,* 1987; Navarro *et al*., 2000
	A. bisporus	Dufour, 1839 and White *et al*., 2000
L. bispinalis	*P. ostreatus*	Yang and Zhang, 1987
L. epleuroti	*P. ostreatus*	Yang and Zhang, 1987
L. fucorus	-	Rinkler and Bloom, 1989; Rinker *et al.,* 1989
L. ingénue (Dufour)	-	White *et al*., 2000; Greenslade and Clift, 2004
Lycoriella jipleuroti,	*P. ostreatus*	Yang and Zhang, 1987
Lycoriella jingpleuroti	*P. ostreatus*	Yang and Zhang, 1987
L. mali Fitch (=*L. solani*) (Winnertz)*	*P. ostreatus*	Thomas, 1942; Clift, 1981; Lewandowski *et al.,* 1999
L. multiseta Felt	*Pleurotus* spp., *A. bisporus*	Thomas, 1942; Greenslade and Clift, 2004
Lycoriella pleuroti	*P. ostreatus*	Yang and Zhang, 1987

Lycoriella yunpleuroti	*P. ostreatus*	Yang and Zhang, 1987
L. pauciseta	-	Fletcher *et al.,* 1986
Lycoriella sp.*	-	Kumar and Sharma, 1999
Phoradonta flavipes	-	Huang *et al.*, 1992
Sciara agaria (Felt)	-	Greenslade and Clift, 2004
S. pulla	-	Hussey, 1969
S. tritici	*A. bisporus, A. bitorquis, A. brasiliensis, A. brunnescens, Pleurotus Cystidiosus, P.ostreatus*	Greenslade and Clift, 2004
Family Phoridae		
*Megaselia agarici (=M. sandui)**	*A. bisporus*	Disney, 1981; Deepthi *et al*., 2004
M. bovista	*A. bisporus*	Hussey, 1959
M. flavinervis	*A. bisporus*	Thomas, 1942
*M. halterata** Wood	*A bisporus*	Hussey, 1959; Brown and Marshall, 1985; Bhandari and Singh, 1986; White, 1982 and Navarro *et al*., 2000
M. iriquoiana	*A. bisporus*	Thomas, 1942
M. nigra	*A. campestris*	Hussey, 1959; White, 1982; Brown and Marshall, 1985
M. scalaris Loew	*A. bisporus* and *P. sajor caju*	Greenslade and Clift, 2004
*M. tamilnaduensis**	*P. citrinopileatus*	Mohan and Disney, 1995
Megaselia sp.*	*Pleurotus* sp.	Lewandowski *et al.,* 1999
Family Scatopsidae		
Colobida fuscipes	*P. ostreatus, Pleurotus* spp.	Lewandowski *et al.,* 1999; Jesse, 2005
Family Sphaeroceridae		
Pullimosina heteroneura (Haliday)	*A. bisporus*	Smith and Gupta, 2002
Tapeigaster annulipes Macquart	*A. campestris*	Greenslade and Clift, 2004
T. nigricornis Macquart	*Agaricus* sp.	Greenslade and Clift, 2004
Family Drosophilidae		
Mycodrosophila sp.	*P. florida*	Deepthi *et al*., 2003
Family Cecidomyiidae		
Henria psalliotae Wyat	*A. bisporus*	White, 1982 and Clift, 1986
*Heteropezina cathistes**	*P. sajor caju*	Johal *et al.,* 1992

Heteropeza pygmaea Winnertz	*A. bisporus* and *P. ostreatus*	White, 1982; White, 1992; Clift and Terras, 1995 and Lewandowski *et al.,* 1999
Lestremia cinerea	-	White, 1982
Mycophila barnesi Edwards	*A. bisporus*	White, 1982; Clift and Terras, 1995
M. speyeri	*A. bisporus*	White, 1982
Mycophila sp.	*Pleurotus ostreatus*	Kim *et al*., 1999
Order Coleoptera		
Atheta coriaria	*P. ostreatus*	Lewandowski *et al*., 1999
*Cyllodes whiteii**	*Pleurotus* sp.	Johal *et al*., 1992
Histerid beetle*	*P. sajor caju*	Kumar, 2006
Pleurotobia tristigmata	*Pleurotus* sp.	Ashe, 1990
*Scaphisoma nigrofasciatum**	*Pleurotus* sp. and *A. bisporus*	Deepthi *et al*., 2004; Kumar, 2006
S. tetrastictum	*P. ostreatus*	Mazumder *et al*., 2008
Scaphisoma sp.* Leach	*P. ostreatus*	Mazumder *et al*., 2001
Staphylinus sp.*	*Pleurotus* sp.	Asari *et al.,* 1991; Deepthi *et al*., 2004; Kumar, 2006

2.1.2 Incidence of collembola (springtails) associated with mushrooms

Species	**Mushroom species**	**References**
Achorutes armatus Nic. (*Hypogastrura* sp.)*	*Pleurotus eryngii*	White, 1982; Kim and Hwang, 1996; Kumar and Sharma, 1997; Marzo, 1998; Kumar, 2006
Brachystomella platensis Najt and Massoud	*A. bisporus*	Greenslade and Clift, 2004
Ceratophysella denticulate (Gisin)	*A. bisporus*	Greenslade and Clift, 2004
C. engadinensis (Bagnall)	*P. ostreatus, Agaricus* spp.	Greenslade and Clift, 2004
H. armata (Nicolet)	*P. ostreatus, Agaricus* spp.	White, 1982; Greenslade and Clift, 2004
H. denisana	*Agaricus* spp.	Sawahata, 2005
H.manubrialis (Tullberg)	*P. ostreatus, Agaricus* spp.	Greenslade and Clift, 2004
H. vernalis (Carl)	*P. ostreatus, Agaricus* spp.	Greenslade and Clift, 2004

Lepidocyrtus sp.*	*Pleurotus* sp. *A. bisporus*	Bahl *et al.,* 1981; Thapa and Seth, 1983
*Lepidocyrtus cyaneus**	*Pleurotus* sp. *A. bisporus*	Bhandari and Singh, 1983; Kumar, 2006
L. ramose	*Pleurotus* sp.	Mignucci *et al*., 2000
Onychiurus ambulans (Linn.)	*P. ostreatus* *Agaricus* spp.	Greenslade and Clift, 2004
O. folsomi (Schaffer)	*P. ostreatus* *Agaricus* spp.	Greenslade and Clift, 2004
Proisotoma minuta (Tullberg)	*P. ostreatus*	White, 1982; Lewandowski *et al.,* 1999
Proisotoma tenella (Reuter)	*P. ostreatus*	Greenslade and Clift, 2004
*Seira iricolor**	*Pleurotus* sp. *A. bisporus*	Gill and Sandhu, 1994
Xenylla mucronata Axelson	*P. ostreatus* *Agaricus* spp.	White, 1982; Greenslade and Clift, 2004
X. welchi Folsom	*P. ostreatus* *Agaricus* spp.	Greenslade and Clift, 2004
Xenylla sp.	*A. bisporus*	Bahl *et al*., 1981
Willowsia sp.	*P. ostreatus*	Lewandowski *et al*., 1999

*Incidence reported from India, - Mushroom species not specified

2.2 Biology and behavior of dipteran flies

Among dipterans, sciarids (Family Sciaridae), phorids (Family Phoridae), cecids (Family Cecidomyiidae) and sphaerocerids (Family Sphaeroceridae) have been found to be associated with cultivated mushrooms.

2.2.1 Sciarids (Diptera: Sciaridae)

Sciarids are small to medium sized delicate flies of smaller to medium size, often bearing resemblance to gnats and midges and are exceedingly numerous in individuals and species. In nature sciarids inhabit leaf mould, wild fungi and rotting vegetable matter. These are the major pests of mushroom throughout the world. Sciarids are initially attracted to the fermentation odors being emitted during cool down of peak heated compost (Richards and Davies, 1977). Different sciarid genera infesting mushrooms show slight variations in their behavior and biology.

Snetsinger (1971) studied the life cycle of sciarids. He observed that an adult female fly laid about 100-170 eggs singly or in the clusters in the spawned compost and casing material of mushroom beds. Freshly laid eggs were round to oval in shape and measured 0.7x 0.3mm in size. Number of eggs laid was found to be temperature dependent. At 35^{o}C, no eggs were laid. Lin *et al*. (1977) observed 95 per cent viability of eggs.

2.2.1.1 *Bradysia* spp.

Shandilya *et al.* (1975) while observing the population of *Bradysia paupera* in mushroom house at Solan (H.P.) reported that its adults were 4.0mm long with dark and long antennae. Larvae measured 6-7mm in length.

Sandhu and Brar (1980) observed that a single female of *B. tritici* laid about 110 eggs in clusters, sometime single or in chains. The eggs were oval in shape and hatched in 7 days at 23^{o}C. Egg viability was up to 95 per cent. Larvae fed on mycelium, mushroom sporophores and decaying organic matter, and became full fed in 8-9 days. The full grown larvae stopped feeding and crawled into the casing surface by moving its abdominal tip. Pupal period lasted for 4-5 days. The adults lived for 2-3 days, The males live longer than females. The life cycle was completed in 24 days at 22.5^{o}C. Five generations of insect were recorded during the cropping period of four and a half month. (Brar and Sandhu, 1989). During summers flies bred in damp, cool places and the larvae fed on moist organic matter. The female flies of *B. tritici* laid eggs mostly in the spawned compost and casing material of the mushroom beds (Brar and Sandhu, 1990).

Shivanna *et al*. (2003) studied the biology of *B. tritici* and found that the eggs were pulpy, white, oval, not visible to the naked eyes and measured 0.23±0.01, 0.12±0.01mm in length and breadth with range of 0.22-0.25 and 0.10-0.14 mm, respectively. The mean egg viability varied from 75-90 per cent. The larval period varied from 9-11 days at 24.5±1^{o}C. Pre-pupal period varied from 1-2 days. The color of pupa was dirty white, which changed to yellowish after 2-3 days of pupation. The pupal survival rate was 80-95 per cent. The adult flies were grayish black with elongated abdomen, long legs and wings. There were fourteen annuli in the flagellum of the antennae.

2.2.1.2 *Lycoriella* spp.

Binns (1980) studied the behavior of *Lycoriella auripila* and observed higher population of the insect in casing as compared to the compost throughout the post casing phase of mushroom culture. Natural population in mushroom house comprised of a mixture of monogenic and digenic strains. Sex ratio of 1.06 females per male was recorded. Males matured earlier than females at 20^{o}C. Females lived longer than males. Wetzel *et al.* (1982) recorded the life span of adult female ≈

five days and of male ≈ three days.

The biology of *L. auripila* as studied by Chakravarty *et al*. (1987) on oyster mushroom revealed that the adult females laid eggs singly or in batches of 2-8 on mycelium mat or at the base of the stipe. A single female laid 130-160 eggs and incubation period lasted for 2-4 days. The larvae tunneled through the fruiting body. The size of *L. auripila* larvae ranged between 6.0-8.0 mm. Before pupation, the larva made silken cocoon and pupated in substrate medium or inside the tunnel in sporophore. Larval and pupal period lasted for 14-19 and 3-6 days, respectively. Adults measured 2.3-2.5 mm in length.

Lewandowski *et al*. (2004) measured the length of eggs, body of each larval instar, pupae and adults. The head capsule of larva was also measured. Larval body length increased from 0.782 mm in first instar to 5.519 mm in fourth instar. Females had wider abdomens; similarly, pupae of females were considerably wider than those of males. Head capsule increased 1.45 times between the instars.

Jesse (2005) recorded that adults of *L. mali* were about 2mm with long thread-like antennae. Larvae measured 6-12mm in length and had a well developed black head capsule. Larvae fed on mycelia, small pinheads, and large mushrooms. Such feeding resulted in cuts in the mycelium, less primodium formation, and cavities in the stipes and caps of large mushrooms. Adult female laid 100-130 eggs at a time on cropping beds and the eggs hatched after 4-5 days at 20^{o}C. Growth and development of the fly was delayed or poor when temperature was lower than 15^{o}C or above 30^{o}C.

Connor and Keil (2005) observed that females of *Lycoreilla mali* were initially attracted to the fermentation odor given off during the cool down period after peak heat of compost. Eggs were laid either singly or in clusters of 8-10, in casing and on the gills or basal stipe of mushroom. Fecundity of 150-170eggs/female was reported. Incubation period of eggs was about 6 days at 18^{o}C. The legless larvae with the shiny black head and powerful chewing mouth parts developed through four instars. Before pupation the larvae spent two days constructing a silken cocoon. Adults emerged on the surface of mushroom beds. The average life cycle is completed in 28 days at 20^{o}C. The adult males prefer to run rather than fly on the casing surface. Mating took place as soon as female emerged from the pupal case.

2.2.2 Phorids (Diptera: Phoridae)

Hussey (1959) discovered the eggs of *Megaselia nigra* attached apically to the gills of

Agaricus campestris. Eggs hatched within three days at 18^oC. The larvae fed on the gills and cap tissues for four days. Pupal stage lasted for five days and life cycle was completed in twelve days. Males on an average lived for 2.4 days and females for 3.0 days. On the other hand, *M. halterata* laid elongated eggs in the casing and compost which measured 0.5 X 0.2mm. Fecundity was 40-60 eggs. The eggs hatched rapidly within two days. Larval period was 9-10 days and pupation occupied further 14 day so that the total life cycle completed in 3-4 weeks.

Sandhu and Bhattal (1986) studied the life cycle of hump backed phorid fly, *Megaselia agarici.* The adults were light to dark brown and 1.9-2.0 mm long. They had inconspicuous antennae and faint sub costa. The tip of the male abdomen was slightly curved downwards whereas in females, it was little swollen, pointed and straight at the tip. Freshly laid eggs were 0.33 mm long, white, elongated, cylindrical and slightly curved. The full grown larvae were dirty white and transparent with visible black mouth hooks. The fully developed pupa was light to dark brown with a pair of black respiratory horns on the thorax. Sandhu and Bhattal (1988) reported that the flies development from egg to adult emergence took 14.6 days at 25.3^oC. The eggs, larval and pupal periods were completed in 1.6, 4.6 and 7.3 days, respectively.

Scheepmaker *et al.* (1997) in his experiments conducted to examine the location of oviposition by the phorid fly *Megaselia halterata* observed that in uncased compost, the majority of the gravid females chose oviposition sites directly after entering the top layer of the compost and 60% of all adults emerged from the top of four compost layers of equal thickness. When the compost was covered by a casing layer which was still uncolonized by *Agaricus bisporus*, oviposition was further concentrated in the top compost layer and 91% of all adults emerged from the top compost layer whereas only 1.5% emerged from the casing. When the casing layer was colonized by mushroom mycelium, 45% of all adults emerged from the casing layer and 53% emerged from the top compost layer.

Megaselia tamiladuensis adults were 2-4 mm long and made quick hopping movements on the substrate. Larvae were 4-6 mm long with a white and transparent body and lacked a distinct black head. Larvae fed on mycelia and made cavities in mushroom fruiting bodies. They usually occured during summer cultivation and caused less damage than other flies (Jesse, 2005).

Studies carried out on the biology of phorid fly, *Megaselia agarici* on white button mushroom, *A. bisporus* by Mrig *et al.* (2006) revealed that its eggs were cylindrical, whitish and measured 0.37mm. The newly hatched maggot was transparent and measured 0.56mm in length. Full grown maggot was dirty white in color with black mouth hooks and measured 3.48mm. Pupa was yellowish brown, bore respiratory horns on thorax and measured 2.37mm. The length of male and female fly including wings was 1.96mm and 2.04mm, respectively. The adults flew 'in copula' in morning and evening hours. The mating, pre-oviposition and post-oviposition periods were 3.6 minutes, 12.8 and 56 hours, respectively. The male and female longevity were 2.7 and 3.3 days, respectively. Spawned compost was preferred for egg laying and fecundity was approximately 43 eggs. The life cycle was completed in 14.9 days at 24^{o}C.

2.2.3 Cecids (Diptera: Cecidomyiidae)

Johal and Kaushal (1991) studied the life cycle of cecid, *Heteropezina cathistes*. They observed that young larvae fed on growing mycelium, tear the bundles of hyphae and fed on exuding sap. The mother larva produced about 12 young larvae and it took 5-7 days to complete one generation. Paedogenesis is the normal mode of reproduction. The larvae crawl on the surface with the help of locomotary pads but on dry surface, the movement is by jumping.

Cecid flies were rarely seen as the larvae reproduced paedogenetically - i.e. new generations were produced within the body of a 'mother' larva without sexual reproduction - and the flies were very small. The larvae were obligate mycelial feeders like phorids. A new generation was produced every 4 to 7 days and each 'mother' larva could produce up to 12 new larvae so numbers increased exponentially. In case of severe infestation, the larvae clumped together and flew over the sides of the `mushroom beds onto the floor where they could be inadvertently transported to healthy crops on feet and equipment (White and Smith, 2000).

Adult cecids of *Mycophila* sp. **(Plate 1)** were less than 1 mm in size and so were difficult to be observed inside the growing room. Larvae were 1-3 mm in length, sucked the nutrients from hyphae and also attacked mushroom stipes and caps. Larvae populations increased rapidly within a short time because they reproduced by paedogenesis during which each larva released 14-20 daughter larvae every 6 days. Mushroom bags/ beds looked orange in color when huge large numbers of orange colored larvae were encountered (Jesse, 2005).

2.2.4 Sphaerocerids (Diptera: Sphaeroceridae)

Sphaerocerids are dark brown flies of 0.5 to 4.0 mm length, very similar in appearance to phorids with the humped body and short antennae but with red eyes. Biological studies of

Pullimosina heteroneura revealed that the adults mated immediately after emergence. Eggs were laid 5-19 days later. Eggs hatched after 24-48 hours into the white larvae. The larvae were 1-5mm long with twelve segments and had three larval instars. Life cycle was completed in 7-18 days and adults lived for several weeks (Smith and Gupta, 2002).

2.2.5 Scaptosids (Diptera: Scaptosidae) (Plate 2)

Scaptosids flies (*Coboldia fuscipes*) were found to occur during summer crop cultivation. Larvae fed on the mycelium, causing rotting of substrate which resulted in yield loss. Larvae grew and developed fast at or above 25^{o}C, but their growth and development was delayed at the temperature below 20 ^{o}C (Jesse. 2005).

2.3 Phoretic behavior

The first available report of phoretic behavior was from Richardson and Chanter (1979) who observed dissemination of *Howardulla husseyi* by its host *M. halterata*. Mushroom mite, *Scutacarus baculitarsus* Mahuka (Acarina: Scutacaridae), was found in clusters on the abdomen of adult flies of *M. halterata* infesting mushrooms (Binns, 1980a). He observed higher number of closely clustered mites on the phorids found in the surroundings of the mushroom houses than on the phorids hibernating in large numbers in the actual culture beds. Phoresy by mites has also been reported by Kumar and Sharma (1998) who reported *Histiostoma* sp. to cling to the legs and thorax of *Megaselia* sp.

As per the observations of Chandran (2000) since the nematodes preferred the inner mycelium layers with dense network, their phoresy reduced to some extent as the flies remained on the surface. However, their tendency to congregate on the surface when present in huge populations ensured phoresy in presence of insects. In his experiment on phoresy by nematodes he recorded 1.0-5.2 nematodes per mushroom fly that included both saprophages and myceliophages. These were found clinging on the legs of dipteran flies caught from the cropping chambers of the mushroom units. Clift *et al.* (2001) reported phoresy by mite *Microdispus lambi* on various species of adult mated dipteran flies associated with cultivated mushrooms.

Kumar (2006) found both nematodes and mites clinging to the legs, thorax and abdomen of adult insects prevalent in the cropping rooms. Mites were found to be more phoretic than nematodes. Per cent phoresy of these organisms was more in beetles as compared to flies.

a. Adult

b. Larvae erupting from the mother' larva

Plate 1 *Mycophila* sp. (Diptera: Cecidomyiidae)

a. Adult

b. Larva

Plate 2 *Coboldia fuscipes* (Diptera: Scaptosidae)

2.4 Seasonal abundance of dipteran flies in mushroom farms

The literature available on this aspect is very scanty as only a few researchers have so far conducted studies on this aspect.

Krishnamoorthy *et al*. (1991) noticed an unusual occurrence of phorid flies (*Megaselia* sp.) in June to September during 1989 in spawn running rooms and beds of oyster mushrooms. According to Kumar and Sharma (1999) sciarids were distributed in mushroom farms all the year round. Under Solan conditions, peak population of sciarids was observed during the month of February followed by March and April when temperature ranged between 11-20^{O}C. Very low population of sciarids was observed during July to October at the temperature ranging between 18-26^{O}C.

Navarro *et al*. (2000) recorded highest number of dipteran flies (Sciarids and Phorids) in the mushroom farm in spring but phorid population increased in autumn. A decrease in the population was observed during the winter season.

Studies conducted by Kumar and Sharma (2001) on the seasonal abundance of mushroom pests with the help of light traps revealed no trapping of sciarids during the month of July, August and September (18 to 25°C). Similarly, phorids were not trapped during the months of February, March and April (11.2 and 20°C). Sciarids started appearing in the month of October and thereafter population gradually built up in following months with peak populations during the months of March, April and May. Phorids started appearing during the months of May, June and in the following months population increased gradually with the peaks occurring during July to October (18.6 and 25°C).

Phorid (*Megaselia* sp.) population was found to be positively correlated with the maximum temperature and was negatively correlated with evening Relative Humidity in oyster mushrooms (Deepthi *et al*., 2004). Kumar *et al*. (2008) reported that in the beginning of mushroom season the number of sciarid flies was higher but in later periods phorid flies were higher. With the increase in temperature from 13.8 to 21.3^{o}C, the number of sciarid fly decreased. On its contrary the population of phorid fly increased very rapidly reaching to 1125 flies in 13th standard week.

Mazumder *et al*. (2008) studied the seasonal abundance of the beetle *Scaphisoma tetrastictum* Champ and reported the incidence from March to November revealing the highest number (12.35) of adults per fruit body and per cent infestation of 29.96 per cent during July. The mushroom remained free from the beetle incidence during the winter months. Highest population and per cent infestation were observed during monsoon (June to August).

2.5 Damaging Potential of insect pests

2.5.1 Sciarids

Though sciarids are the pests of most common occurrence in the mushroom farms, the literature available on their damaging potential is scattered and sparse.

Zaayen (1978) reported that sciarid could cause more damage than phorids. Crop reduction to the level of 50 per cent was recorded due to these pests in Holland.

Under Punjab conditions in India sciarids were known to cause 66.7 per cent infestation (Sandhu and Brar, 1980).

Kielbasa and Snetsinger (1981) reported that the greatest loss in yields occurred in the second and third flushes of *A. bisporus* and a population density equivalent to 13 larvae/sample was the injury threshold for *L. mali.* Similarly, White (1986) observed maximum reduction of 8 per cent in the yield of *A. bisporus* during second and third flush when a mean of 10 sciarid (*L. mali*) larvae/125 g sample were inoculated.

Goltapeh (1991) observed that the larvae of sciarids entered the mushroom from the casing material through the stipe in large numbers started feeding. As a result of feeding, tunnels were formed within the stipe. Eventually they reach the pileus and fed vigorously on the gills. Larval attack at pin head formation time resulted in cessation of pin development. During maturity larvae left the tunnels and returned to casing material for pupation. The infested mushrooms turned yellowish brown, rotten and became unfit for consumption.

2.5.2 Phorids

Rinkler and Snetsinger (1984) studied the damage threshold of *Megaselia halterata* on a commercially cultivated mushroom, *Agaricus brunnescens*. They observed the population of 1900, 3110, 9800, 12443 and 12776 per m^2 of growing surface during spawn run. However, mushroom yield was not significantly reduced by infestation levels of 1900, 3110 or 9800 females per m^2 of production surface. The levels of 12,443 and 12,776 per m^2 significantly reduced yields by first break and continued through the fifth break.

Sandhu and Bhatal (1988) reported 10.52-73.30 per cent infestation by *M. sandhui* during March-April in Punjab.

Aggarwal (2000) revealed an infestation of *M. sandhui* in the range of 8-92 per cent during March that resulted into total crop destruction in April in a mushroom farm in Haryana. Kumar and Sharma (2000) reported 46 per cent loss in yield of button mushrooms due to phorids. In oyster mushrooms, particularly during rainy season, phorids could cause 100 per cent loss in yield.

2.5.3 Cecids

White (1990) reported that introduction of the mushroom cecid, *Heteropeza pygmaea* into a mushroom crop at rates of 2, 20 or 200 larvae/tray (0.56/m^2) caused significant reduction in both the yield and number of mushrooms in relation to the infestation level. The reduction was maximum when the larvae were introduced at time of spawning. Casing time infestation of the insect caused lesser damage. Just two larvae introduced at the time of spawning resulted a loss of 12 per cent in total yield, in addition to 7 per cent loss due to spoilage.

A study on damaging potential of three species of paedogenetic cecidomid on *A. bisporus* revealed the *M. barnesi* was the most damaging species followed by *H. pygmaea and Henria psalliotae* (Clift and Terras, 1995).

2.5.4. Economic Threshold Level

White (1986) reported that economic threshold level for mushroom sciarid, *Lycoriella auripila* was virtually zero making chemical control necessary at very low larval densities. Generally, infestation by the pest during spawning or at spawn running time resulted in greater yield losses than infestation after casing (Kumar *et al*., 2001). The economic threshold level of the sciarid pests, above which economic loss would occur, has been presented in summarized way in herein.

Economic threshold level of Sciarid flies for various aspects at different times and locations on mushroom farms

Pest	Situation	Economic threshold level	Comments
Sciarids	Out side spawning/ casing area	> 5 adults per week on trap	These adults have either flown in from elsewhere or are escapes from older rooms
	Spawn run	Any live adult	As above
	< 2 weeks after casing	Any live adult	These are the first generation adults from infestation at spawning or cool down
	First flush	Any live adult	First generation from infested casing
	> 2nd flush	> 20 per trap per Week or 10-201ive adults per room	Limited control possible of larvae in beds. Adults should be restricted to reduce reinfestation

Source: Clift and Terras, 2000

2.6 Biomanagement

Mushrooms are highly sensitive and are often consumed fresh immediately after harvest. Moreover, they have a unique cropping pattern in which fruiting bodies appear in breaks of 6-8 days in a short duration crop of 6-8 weeks. These characteristic features make the use of chemical pesticides in this crop nearly practically impossible. Thus, necessity to control its insect pests by using eco-friendly bio-management practices wherein certain fungi, bacteria, entomophagous nematodes, predatory mites and plant products are used is emphasized.

2.6.1. Entomophagous nematodes

Richardson (1987) studied the potential of two species of entomophilic nematodes, *Steinernema feltiae* (=*Neoaplectana carpocapsae*) and *Heterorhabditis heliothidis* for biological control of mushroom flies in pot trials. The larvae of the phorid, *M. halterata,* the cecidomyiid, *H. pygmaea* and the sciarid, *L. auripila* were all susceptible to parasitism by both nematode species. Fewer adult phorids and sciarids emerged when nematodes were in the compost. Casing treatments were more effective than spawning treatments; little extra benefit was gained by applying the nematodes twice. Populations of paedogenetic larvae of *H. pygmaea* built up rapidly in untreated compost but were reduced when *N. carpocapsae* was applied, and were eradicated by *H. heliothidis,* because they could penetrate insect cuticle, as well as through natural body openings. Thus, *Heterorhabditis heliothidis* were more suitable than *Neoaplectana* spp. for the control of mushroom fly larvae.

Nickle and Cantelo (1991) studied the relationship between numbers of *S. feltiae* added to the compost @ 0, 69, 207, 310 and 620 IJs/cm^2 and the control achieved against the third instar larvae of *L. mali.* Approximately 100-200 eggs from the infected compost were extracted and fly emergence was observed with the help of sticky pots label placed on the compost to catch emerging flies. Minimum fly emergence (15%) was observed with the dose of 620 nematodes/cm^2, whereas, maximum fly emergence of 45 per cent was observed with the dose of 69 nematodes/cm^2.

According to Taylor (1992) *S. feltiae* application was highly effective in controlling larval population of *Lycoriella* sp. and lasted longer than diflubenzuron. Grewal *et al*. (1992) observed increase in the mean total number (28.5%) and weight (19%) of fruiting body of *A. bisporus* with the application of *S. feltiae* at a rate of $3x10^6$ IJs per tray (surface area=0.56 m^2). Grewal *et al*. (1993) obtained the effective control of maggot population of *Lycoriella* sp. by applying strains ScP and SN of *Steinernema feltiae* ($0.5x10^6$ and $1.0x10^6$ infective juveniles/m^2 cropping area). Scheepmaker *et al.* (1995) effectively controlled the F2 generation of *L. auripila* by late casing treatment with *S. feltiae* at a concentration of $5x10^3$ infective juveniles/m^2.

Rovesti *et al.* (1996) observed that *S. feltiae,* significantly reduced the number of adults of *Lycoriella* sp. emerging from mushroom compost and its effectiveness lasted for at least 3 weeks. The treatments also reduced the number of damaged sporocarps, although no differences were recorded in the yield of mushrooms.

Bioassays were initially conducted by Scheepmaker *et al.* (1998a) in Petri dishes to screen the efficacy of four *Heterorhabditis* and *Steinernema* species against the mushroom phorid, *M. halterata.* Control rates of 61-70% were obtained at 1500 infective juveniles (IJs) per 30 fly larvae. In a screening of different species of *Heterorhabditis* and *Steinernema* with applications of 30 IJs per phorid larva, the highest parasitization rate of 20% was obtained with S. *feltiae.* At the lowest dosage of 30 IJs per sciarid larvae, 78% control was obtained. Increasing the dosage from 30 to 1000 IJs per sciarid larvae led to only small increase in phorid mortality. At 1000 IJs per larva, significant mortality of 18% was obtained. The nature of the substrate, compost or casing did not greatly influence the parasitization rates.

Scheepmaker *et al.* (1998b) studied the effect of *S. feltiae* against larvae of the mushroom phorid, *M. halterata* and the mushroom sciarid, *L. auripila* in the Netherlands. Sciarid larvae originating from infestations in casing soil during colonization by *Agaricus bisporus* were almost completely controlled by applications of *S. feltiae* to the casing soil. When larvae originated from infestations in freshly spawned compost, they could be controlled by compost applications halfway through spawn-running and by very early casing treatments. The control of phorids in compost was maximally 31 per cent when nematodes were mixed within the infested compost at a concentration of 3 x 10^6 nematodes/m^2. At concentrations of 6 and 15 x 10^6 nematodes/m^2, *S. carpocapsae* resulted reduction rates of 65 and 73 per cent, respectively, when it was applied 3 days after the end of the infestation period.

Kim *et al.* (2001) studied the potential of entomophilic nematodes, *S. carpocapsae* Pocheon strain and *H. bacteriophora* Hamyang strain against the mushroom fly *L. mali* infesting *P. ostreatus* in the laboratory and in the field. *S. carpocapsae* was more effective than *H. bacteriophora* in controlling the pest. Mortality of *L. mali* was higher at 25^{o}C than at 20^{o}C. The third and fourth instars of *L. mali* were more susceptible than the second instar. *S. carpocapsae* infected all stages except egg and first instar.

Sznyk and Bednarek (2003) studied the efficacy of entomopathogenic nematodes and insect growth regulator Trigard (cyromazine as active ingredient) against sciarid fly, *L. solani* infesting cultivated mushrooms. Combined treatments caused higher mortality in *L. solani* larvae than treatments with nematodes alone. The efficiency of combined treatments appeared similar to the efficiency of treatments with the insecticide at the recommended concentration.

Kim *et al* (2004) tested the Korean isolate of *S. carpocapsae* Pocheon strain against fungus gnat, *B. tritici* under the laboratory conditions. *S. carpocapsae* affected oviposition, with the untreated females laying an average of 121±25 eggs, whereas the treated females averaged 7±2 eggs. The infectivity of *S. carpocapsae* to the fungus gnat was affected by the developmental stage and temperature, with highest mortality observed with the third and fourth instars and pupal stage. Nematode mortality in the second instar fungus gnat ranged between 23 and 35%.

Walia *et al*. (2004) conducted trial for the management of *M. sandhui* by *Steinernema* isolates (10^6/m^2) under laboratory conditions and reported 30-40 per cent mortality of maggots after 72 hours. Jess and Bingham (2004) observed that entomopathogenic nematode, *S. feltiae* (Filipjev) applied at 3x10^6 nematodes/m^2, controlled sciarids and phorids in mushroom compost and casing substrates. A majority of S. *feltiae* nematodes resided at a depth of 24 cm in both substrate types.

In *vivo* experiments conducted by Lamba *et al*. (2007) using three levels of *H. indica* revealed lowest infestation by *M. sandhui* (5.3 %) in case of 10x10^4 IJs/5kg compost, followed by 5 x10^4 IJs/5kg compost (7.1%) treatment of *H. indica*. Highest yield was obtained with 10x10^4 IJs, which was at par with 5 x10^4 IJs. Efficacy of *S. carpocapsae* and *H. indica* was tested by Gitanjali (2008) against larvae of sciarid fly *Bradysia* sp. under laboratory conditions in *P. sajor-caju*. *Heterorhabditis indica* caused higher mortality (70%) of larvae of *Bradysia* sp. than the *S. carpocapsae* (56-60%). The nematodes had no significant effect on mycelial growth of *P. sajor-caju*.

2.6.2 Predatory mites

Al-Amidi *et al*. (1991) studied the predatory potential of mite, *Parasitus bituberosus* and observed 50-60 per cent reduction in larval population of *L. solani*. A list of predatory mites that feed upon sciarids has been provided hereunder.

Predatory mites on mushroom sciarids

Mite	**Host**	**Reference**
Hypoaspis miles	*Bradysia* spp., *L. auripila*	Wright and Chambers, 1994
Geolaelaps aculeifer	*Bradysia* spp.	Gillespe and Quiring, 1990
Parasitus fimetorum	Sciarids	Binns, 1973
Arctoseius cetratus	Sciarid larvae and eggs	Binns, 1973
Linopodes antennaepes	Sciarid larvae and eggs	Al-Amidi and Downes, 1990
Parasitus bituberosus	*L. solani*	Al-Amidi *et al*.,1991

2.6.3 Fungal and bacterial bioagents

Rovesti *et al.* (1996) observed that *Bacillus thuringiensis* subsp. *israelensis* and azadirachtin significantly reduced the number of adults of *Lycoriella* sp. emerging from mushroom compost and effectiveness lasted for at least 3 weeks. The treatments also reduced the number of damaged sporocarps, although no differences were recorded in the yield of mushrooms.

Bhat *et al*. (1998) evaluated the efficacy of *Bacillus thuringiensis* and Neem formulations Rakshak and Neemark against dipteran pests infesting oyster mushroom and compared them with endosulfan. Among the biopesticides, spray application of Rakshak @ 0.15 per cent resulted in the lowest fruit body infestation (1.97%). However, in the first week *B. thuringiensis* @ 0.5 per cent, Neemark @ 0.03 per cent and Rakshak @ 0.15 per cent were at par with untreated control. No infestation of pests was observed throughout the cropping season in endosulfan treated bags. Highest yield was obtained from application of endosulfan at spawning.

2.6.4. Resistant strains

Twelve isolates from the genus *Agaricus* (Fungi, Basidiomycota) were investigated for their susceptibility/resistance against the phorid fly, *M. halterata*. Combined effects of oviposition of adult female *M. halterata* and larval development in mushroom compost inoculated with *Agaricus* mycelium were determined using bioassays. The numbers of *M. halterata* offspring that developed were affected by the *Agaricus* isolate used, and there was a significant separation between resistant and susceptible isolates. In a bioassay where the female phorids had a choice of all 12 isolates for oviposition, three isolates produced >200 adults per 100 g compost pot while the remaining nine isolates had <20 adults per pot. Where there was no choice of *Agaricus* isolate for oviposition, five isolates resulted in >100 adults per 100 g compost pot while the remainder resulted in <4 adults per pot. With the susceptible isolates, there was a positive correlation between increasing concentration of mycelium in the substrate and phorid development until the concentration exceeded 40% after which numbers of emerging phorids declined. Species of *Agaricus* with resistance to *M. halterata* could have significant potential for the breeding and cultivation of phorid-free mushrooms (Smith *et al.*, 2006).

2.6.5 Plant products

Bhat *et al.* (1998) studied the effect of various Neem formulations against insect pests of oyster mushroom. They observed that among biopesticides spray application of Rakshak @ 0.15 per cent resulted in the lowest fruit body infestation (1.97%). Application of Rakshak and Neemark to the substrate increased the yield by 42 and 28 per cent, respectively, over control.

Park *et al.* (2005) studied the effect of fumigation toxicities of essential oils and monoterpenes against *L. mali* adults. The most potent fumigant toxicity was found in essential oils from thyme followed by the oils of sage, eucalyptus and clove bud. A-Pinene was most toxic fumigant found in thyme oil (LDso=9.85µl/1 air) followed by β- pinene (LD_so=l1.85 µl/1 air) and linalool (LD_so=21.15 µl/1 air).

CHAPTER-3

MATERIALS AND METHODS

The present research on the subject entitled "Studies on incidence, pathogenicity, biology and biomanagement of insect pests associated with cultivated mushrooms" was conducted in the Department of Entomology and Apiculture and Mushroom Research Laboratory of Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan during the years 2006-09 as per the methodology given below:

3.1 Faunistic survey and identification of insect-pests and collembola associated with cultivated edible mushrooms

The insect and Collembola fauna associated with cultivated edible mushrooms were collected from the mushroom units growing *Agaricus* spp. and /or *Pleurotus* spp. located in various districts of Himachal Pradesh viz., Bilaspur, Hamirpur, Kangra, Kullu, Mandi, Shimla, Sirmaur, Solan and Una. Samples from pasteurized and unpasteurized compost, casing material, spent compost, spawned compost, cropping beds, platform soil and sporophores were collected and analyzed to record the incidence of insect fauna. Also, the adult dipteran flies hovering on the cropping bags and coleopterans feeding on the fruiting bodies were collected/ picked. Faunistic studies could not be conducted in the districts of Chamba, Lahul Spiti and Kinnaur as no mushroom unit was operating at commercial level in these areas.

3.1.1 Collection and identification of insect fauna

Samples for immature stages of insects were collected from above referred composting ingredients as well as cropping beds in fresh polythene bags, sealed properly with rubber bands to avoid moisture losses and labeled for locality, cropping time and date of collection. Samples brought to laboratory were processed for studying the incidence of insects and springtails without undue delay. Precaution was taken to maintain optimum moisture level until the analysis was complete.

Adult insects hovering over the cropping bags/inside the mushroom farms were collected by different methods, with or without net only for identification purpose. Dry and wet collections (70% alcohol) of the captured stages were prepared. Identification was done by the use of taxonomic keys and was got confirmed from insect taxonomists of concerned field. Yellow traps were laid in the cropping rooms of three farms located in near by vicinity of Maryog, Jatoli and Nauni University farm for seven days to have an idea of adult insect population in the cropping rooms. 'Pest O Lure' (Pest Control (India) PVT. Ltd.) traps were used to trap the adult flies. In all, twenty three farms located in nine mushroom cultivating districts of the state were covered during the tenure of these studies.

3.1.1.1 Collection of adult dipteran flies

Dipteran insects were collected with help of yellow traps. Yellow bulb of 15 W was placed in between two polythene sheets (supported by card board) of 12 cm x 15 cm size coated with mustard oil among the bags at a height of 60 cm from the ground. The bulb was switched on from 5 p.m. to 8 a.m. Polythene sheets with insects sticking to them were brought to the laboratory once in a week and the insects were identified and counted separately.

3.1.1.2 Collection of coleopteran insects

The beetles and their grubs were collected from individual sporocarp and the mean number of beetles/grubs per sporocarp was calculated. Staphylinid beetles, cyllodes and other beetles were collected from 250 cc. compost/casing soil and the infested fruiting bodies in case of *A. bisporus* and from substrate (250cc.) and sporocarps in case of *P. sajor* caju. The collected fauna were preserved separately in 70 per cent ethyl alcohol for identification. Identification was done with the help of taxonomic keys and was got confirmed from insect taxonomists.

3.1.1.3 Extraction of springtails and other arthropods

A plastic funnel with a piece of rubber tube (10 – 12 cm) bearing a glass vial of 5 ml capacity attached to its distal end was taken. Funnel was fixed with funnel stand and a molded piece of wire net was placed over the funnel. The substrate sample with expected fauna was placed over the wire net gently. A table lamp was fixed over the sample to provide heat and light. The prevalent fauna started moving downward due to their sensitivity to heat and light and was collected in the glass vial.

The collected fauna were preserved separately in 70 per cent ethyl alcohol for identification.

Identification was done with the help of taxonomic keys and was confirmed by insect taxonomists.

3.2 Seasonal Abundance of dipterans

Seasonal abundance of test insect viz., *Sciara* sp. was recorded by taking monthly observations regarding the presence of these flies on *A. bisporus* and *Pleurotus sajor caju* cultivated in the selected mushroom farm located at UHF, Nauni, Solan. Data were collected from August 2007 to December 2009. Pest-O-Lure yellow sticky traps were used to capture the insects. Traps were observed at weekly intervals during evening hours. Traps to which flies were sticking were brought to the laboratory and the flies were counted. Traps were operated throughout the year irrespective of the mushroom species grown.

3.2.1 Studies on Phoretic behavior of insects

Samples of adult insects, each consisting of five individuals of a particular genus were collected separately from one mushroom unit each located in seven districts working under unhygienic conditions in make shift rooms which normally harboured nematodes/mites/ other arthropod pests. The samples were brought to laboratory and the individual insects were washed thoroughly in small quantity of water. The wash water was further observed under microscope for the presence of mites/ nematodes/other tiny arthropods if any. The number of mites/ nematodes found in each sample of wash water represented their phoretic intensity. Per cent frequency of phoresy was also worked out.

3.3 Isolation and raising of pure culture of test insect

Sciarid flies belonging to Order Diptera (Family Sciaridae) were found in small to large numbers in most of the farms surveyed. The farms harboring huge populations of these insects generally produced poor yields of fruiting bodies. Thus, these flies were selected as the test insect for further investigations. The mushroom beds showing visible fauna of immature/mature insects were selected for sampling. Compost/substrate sample of 250 cc were collected from the cropping bags of the test mushrooms separately. These samples were analyzed for the presence of insect larvae under the laboratory conditions. The larvae were hand picked under the stereoscopic microscope/ or with naked eye and inoculated in Petri plates fully impregnated with mycelium of the test mushroom grown on malt extract agar medium. The culture thus raised, was maintained, multiplied and used for further experiments. Malt extract agar medium was used to maintain the culture of *A. bisporus* and Potato dextrose agar was used for *Pleurotus sajor-caju*.

3.3.1. Mass culturing of sciarid flies

Mass culture of sciarid flies was maintained under the farm conditions on 10 kg capacity bags of white button mushroom and oyster mushrooms. Ten bags each for the test mushrooms were used for mass culturing. Larvae were inoculated into these bags from the laboratory cultures at the time of spawning. These were allowed to feed upon the growing mycelium and to complete their life cycle. The adults thus emerged form the inoculated larvae mated and females laid eggs on the compost/ substrate of the test mushrooms which helped in the mass multiplication of these flies.

3.3.2 Sterilization of glassware

Glassware to be used was thoroughly cleaned with detergent powder, washed under running tap water, followed by rinsing in distilled water. The flasks after pouring the medium were tightly plugged with cotton gauge before autoclaving. Further, the cotton plugs were covered with aluminum foil. Flasks and other glassware were autoclaved at 1.5kg/cm^2 for half an hour and were kept in hot air oven at 180^{0}C temperature for drying for one hour. Laminar airflow chamber was sterilized by ultraviolet irradiation for 30 minutes and with absolute alcohol before its use.

3.3.3 Malt extract agar medium

3.3.3.1 Composition

Malt extract	-	25g
Agar-Agar	-	20g
Distilled water	-	To make final volume 1000ml

3.3. 3.2 Method of preparation

The medium was prepared by melting 25 g of malt extract in 500 ml of distilled water by heating. Twenty gram of agar-agar was added with continuous stirring till all the constituents were thoroughly mixed up and final volume was made to 1000 ml, pH of the medium was adjusted to 6.5. The medium so prepared was poured in conical flasks and these were plugged tightly with non-absorbent cotton and autoclaved at 15lb/inch^2 for half an hour. The medium thus prepared was poured in sterilized Petri plates under aseptic conditions (using the sterilized laminar flow) and allowed to solidify.

3.3.4 Potato dextrose agar medium

3.3.4.1 Composition

Potatoes	-	200g
Dextrose	-	20g
Agar-Agar	-	20g
Distilled water	-	To make final volume 1000ml
pH	-	6.5-7.0

3.3.4.2 Method of preparation

Potatoes were thoroughly washed, pealed and cut into small pieces. The cut pieces were boiled in half litre of water for at least 20 minutes. Extract was filtered through muslin cloth and final volume was made to 1000ml. In this final volume, 20g dextrose and 20g agar-agar were added with continuous stirring so that no clods remained. The medium so prepared was poured in conical flasks and these were plugged tightly with non absorbent cotton. These flasks were autoclaved at 15lb/inch^2 for 15 minutes. The medium was poured in sterilized Petri plates under aseptic conditions and allowed to solidify.

3.3.5 Inoculation and raising of mycelium of test mushrooms on malt extract agar /potato dextrose agar medium

The pure cultures of *A. bisporus* and *P. sajor caju* were obtained from Mushroom Research Laboratory, Department of Mycology and Plant Pathology, UHF, Chambaghat, Solan. Petri plates were autoclaved at 15lb/inch^2 for half an hour and dried in hot oven at 180°C for one hour. The sterilized Petri plates were then poured with sterilized media under aseptic conditions and the media were allowed to solidify. Small uniform bits of mycelium of respective mushrooms were cut with the help of cork borer and one bit each was placed separately at the centre of each Petri plate containing solidified medium under aseptic conditions. After inoculation, the Petri plates of *A. bisporus* and *P. sajor caju* were incubated at the respective temperatures of 23±1^{0}C, and 26±1 ^{0}C for 14 days so as to allow complete spread of mycelium.

3.4 Biology of the prevalent insect species

The biology of *Sciara* sp. was studied under laboratory conditions on white button mushroom, *A. bisporus* as well as oyster mushroom, *P. sajor caju* at respective temperatures of 23±1^{o}C and 26±1^{o}C. Laboratory culture of the fly was maintained separately on the malt extract agar medium Petri plates with fully impregnated mycelium of *A. bisporus* and potato dextrose agar medium plates with full grown mycelium of *P. sajor caju.* Pupae of the test species were collected from the running culture and released separately into number of Petri plates @ 10 pupae/plate (five males and five females) with fully impregnated mycelium of test mushrooms. The emerging flies were allowed to mate. Mating period of newly mating single pairs of adults "in copula" position in Petri plates was observed till the males and female elapsed from copula. The eggs laid were observed under the stereoscopic microscope. Each plate was left with a single cluster of eggs, generally containing 10 to 14 eggs. The eggs were allowed to hatch and various immature stages were observed thrice a day until the adult fly emerged out of the pupa. Magnifying lens (10X) and stereoscopic microscope (20 X) were used to examine different stages of the insect.

3.4.1 Male to female sex ratio

Natural sciarid populations were picked up from five button mushroom growing mushroom farms located at Nauni, Jatoli, Maryog, Sehal and Darin in order to determine male to female sex ratio of the insect. Similar studies were conducted in oyster mushrooms. Population samples were collected from oyster farms located at Nauni, Oachghat, Maryog, and Dumki.

Sex ratio was calculated on the basis of the visible characters of females and males viz. ovipositor in females and claspers in males at the tip of the abdomen. The abdomen was more swollen in females as compared to males.

3.4.2 Morphometrics of sciarid flies

Measurements of different stages of sciarid fly, right from egg to adult were taken with an ocular micrometer using a compound microscope. The different stages were also photographed. In all, ten insects were measured and their average was worked out. Day to day measurements of larvae was recorded.

3.5 Relative susceptibility of the test mushrooms against test insect:

Relative susceptibility of *A. bisporus* and *P. sajor caju* was tested against larvae of sciarid fly (*Sciara* sp.). For undertaking this experiment, 15 Petri plates each with full grown mycelium of

A. bisporus and *P. sajor-caju* developed on malt extract agar and Potato dextrose agar medium, respectively, were inoculated separately @ 2, 4, 8 and 16 sciarid larvae/plate. Each treatment was replicated three times. Uninoculated Petri plates were kept as control. The observations were recorded after 2, 4, 6, 8 and 10 days. These experiments were laid out in completely randomized design (CRD).

Observations regarding mycelial depletion were recorded at 2,4,6,8 and 10 days of inoculation. Since, the sciarid larvae made tunnels in the mycelium, it was not possible to access the mycelial depletion caused by them directly on area basis. So, squares (9x9 mm) were made on the cover of Petri plates inoculated with sciarid larvae. Damaged squares were counted at respective intervals and the percent mycelial depletion was calculated using the following formula:

$$\text{Per cent mycelium depletion} = \frac{\text{Damaged squares}}{\text{Total number of squares}} \times 100$$

3.6 Substrate preparation and production technology of test mushrooms

3.6.1 *Agaricus bisporus* (Lange) Imbach (White button mushroom)

3.6.1.1 Spawning and spawn run

Compost was procured from Department of Horticulture, UNDP Chambaghat, Solan and University Mushroom Unit. Spawn (U-3) supplied by Spawn Laboratory, Chambaghat, Solan (HP) was used in all the experiments. Pasteurized compost containing 68-70 per cent moisture and pH of approximately 7.2 was spawned @ 0.5-0.75% (i.e. 50g to 75g spawn/10 kg compost) on fresh weight basis and filled in polypropylene bags of different sizes as per the requirement of the experiment. After the addition of spawn, the bags were blended such that spawn was thoroughly mixed in the compost. The material was gently hand pressed after spawning to the extent that the bags do not break. Finally, the bags were covered with newspaper sheets dipped in 2.0 per cent formalin (to avoid aerial contamination) and were transferred to spawn run room where efforts were made to maintain optimum temperature, relative humidity, light and ventilation conditions as per the requirements of *A. bisporus* cultivation. Relative humidity was maintained by spraying water on walls and floors of cropping rooms.

3.6.1.2 Casing (Top dressing)

Pasteurized casing soil supplied by Department of Horticulture, UNDP, Chambaghat was used for the purpose. Casing was spread in the form of 4 cm thick layer on the compost with full mycelial spread. Following environmental parameters were maintained:

Parameters	**Spawning period**	***Cropping period***
Temperature	24 $\pm$ 2 ^{0}C	16 $\pm$ 2 ^{0}C
Relative humidity	65 –70 %	85 – 92 %
Light	Nil	Nil
Ventilation	Minimum, so that there was no fluctuation in the temperature.	Forced air circulation is essential.

3.6.1.3 Harvesting

Fruiting bodies were harvested when the sporophores were fully mature, by twisting lightly clock and anti-clockwise so that the young developing fruiting bodies were not damaged.

3.6.2 *Pleurotus sajor caju* Singer (Oyster mushroom)

3.6.2.1 Preparation of substrate and spawning

The chopped wheat straw was soaked in clean tap water for 10 – 12 hours. After draining out excess water, the substrate was put in polypropylene bags and sterilized at 1.5 kg/cm^2 (20 lbs/inch^2) pressure for one to two hours in steam sterilizer. This sterilized substrate was spawned with freshly prepared (20-30 days old) grain spawn @ 2.0-3.0 per cent ((i.e. 200g to 300g spawn/10 kg compost)) on w/w basis and further filled in polypropylene bags as per requirement. After complete mycelium spread (about 20 days after spawning) the bags were reversed and the poly propylene sheet was cut removed. Water was frequently sprayed in the cropping rooms depending upon the atmospheric humidity. The following environmental parameters were maintained in the cropping rooms:

Parameters	*Spawning period*	Cropping period
Temperature	10-30 ^{0}C (Optimum 22-26 ^{0}C)	16-30 ^{0}C
Relative humidity	85 – 95 %	85 – 92 %
Light	Nil	200 lux for 8-12 hours
Ventilation	Sufficient ventilation	Sufficient ventilation during fructification
CO_2	Up to 2000 ppm	Less than 600 ppm

3.7 Damaging potential of *Sciara* sp. on *A. bisporus* and *P. sajor caju*

The experiment was conducted in 10 kg capacity polypropylene bags, each filled with compost/substrate as per the requirements of the test mushroom. Spawning was accordingly done with the spawn of *A. bisporus* and *P. sajor caju* @ 0.5 and 1.0 per cent, respectively. The production technology and other parameters were the same as explained in the earlier experiment (3.6). Three levels of inoculum i.e. 2, 20 and 200 larvae of *Sciara* sp. per bag were inoculated separately used for each *A. bisporus* and *Pleurotus sajor caju*.

Agaricus bisporus bags were inoculated separately with these inoculum levels at the time of spawning, casing as well as at both spawning and casing time. The bags of *P. sajor-caju* were inoculated at spawning, at pin head stages and both at spawning and pin head formation stage. Uninoculated bags were maintained as control. Each treatment was replicated three times and the experiments were laid out under Completely Randomized Design (CRD).

Following observations were recorded:

i. Mycelial growth

Observations regarding per cent mycelial growth were recorded at casing, 15days, 30 days and 45 days of casing and at the termination of the experiment (60 days) in all the treatments and compared with the mycelial spread in uninoculated control bags in *A. bisporus*.

$$\text{Per cent mycelial growth} = \frac{\text{Total surface area - Depleted area (cm}^2\text{)}}{\text{Total surface area of bag (cm}^2\text{) }(\Pi r(r+h))} \times 100$$

Where, r = radius of the bag

h = height of the bag

Mycelial growth in *P. sajor caju* could not be observed as whiteness of the mycelium in this mushroom is not spectacular.

ii. Population of test fly

Samples of 250 cc compost/substrate were picked from all *A. bisporus* growing bags receiving different treatments at different intervals viz. casing, 15days, 30 days and 45 days of casing and at the termination of the experiment and were analyzed for the insect population.

Similarly, the insect population in bags growing *P. sajor-caju* was assessed at plastic removal, 20 and 40 days after plastic removal and at the end of the cropping.

iii. Flush pattern and total sporophore yield

Sporophore yield on day to day basis was taken for each replication of all the treatments as well as uninoculated control from day one of button appearance to the end of the cropping. Since the buttons normally appear in distinct succession of flushes at 7-8 days interval in *A. bisporus,* weekly yields per treatment were pooled to have information regarding the yield per flush. Data on yield per flush for each treatment were finally pooled to quantify the total sporophore yields. Similar observations were recorded for *P. sajor caju* also.

iv. Flush gap periods:

Flush gap period of one replication of each treatment in *A. bisporus* and *P. sajor-caju* was recorded separately.

v. Visual symptoms on sporophore

Symptoms produced by test insect on sporophores of test mushrooms were visualized under microscope as well as with naked eyes. Sporophores were also dissected to observe the internal damage.

3.8 Management of *Sciara* sp.

Experiments for management of *Sciara* sp. in both the test mushrooms were conducted *in vivo* and *in vitro* phase. In all, four experiments were laid wherein two Neem products viz. Neem seed kernel extract (NSKE), Neem seed kernel powder and two entomopathogenic nematodes (EPN) viz. *Steinernema carpocapsae and Heterorhabditis indica* were tested for their efficacy against the test insect.

3.8.1 Preparation of Neem (*Azadirachta indica*) extract

The aqueous extract of Neem seeds was prepared under the laboratory conditions on per cent basis as per the method given by Gahukar (1996) and Sharma *et al.* (1997). Neem seeds were crushed with the help of mixer grinder. Ten per cent stock solution of Neem seed kernel was prepared by mixing 10 gm of crushed neem seeds in 100 ml of distilled water. The mixture was kept overnight with continuous stirring from time to time. Next day, the mixture was passed through the muslin cloth and filtered with the help of Whatman filter paper No. 1. The filterate was considered as stock solution. Further dilutions were made from this stock solution with distilled water by using single dilution method.

$$\text{Quantity of NSKE required (ml)} = \frac{\text{Concentration required (\%)}}{\text{Given concentration (\%)}} \times \text{Vol. of medium to be prepared (ml)}$$

Neem seed kernel powder was prepared by crushing shade dried Neem seeds in the grinder.

3.8.1. *in vitro* experiment

Neem seed kernel extract and Neem seed powder were mixed @ 1.0 per cent in malt extract agar and potato dextrose agar, separately, at the time of media preparation. Each treated medium was poured in 30 Petri plates of 9.0 cm diameter separately under aspetic conditions and was allowed to solidify. Other plates were poured with untreated media. All the plates having malt extract agar medium (both treated and untreated) were inoculated with a bit of *A. bisporus* mycelium and incubated at 20 $\pm 1^{O}$C for ten days. The plates with potato dextrose medium were inoculated with a bit of *P. sajor caju* mycelium and kept at 24 ± 1^{O}C for mycelial impregnation for ten days. Two species of entomophagous nematodes viz. *Steinernema carpocapsae* and *Heterorhabditis indica* were also used as bioagents in the experiment. Once the mycelial spread was complete, newly emerged larv of *Sciara* sp. were inoculated @ 20/plate. Each treatment was replicated three times and statistical analysis was done as per completely randomized design (CRD). The treatment details for each of the mushrooms parasitized by *Sciara* sp.a re given below:

Treatments

Tl = Test mushroom + *Sciara* sp.+ Neem Seed Kernel Extract (1%v/v)

T2 = Test mushroom + *Sciara* sp.+ Neem Seed Kernel powder (1 %w/v)

T3= Test mushroom + *Sciara* sp.+ *S. carpocapsae* (1 x 10^2)

T4= Test mushroom + *Sciara* sp.+ *S. carpocapsae* (1 x 10^3)

T5= Test mushroom + *Sciara* sp.+ *H. indica* (1 x 10^2)

T6= Test mushroom + *Sciara* sp.+ *H. indica* (1 x 10^3)

T7= Test mushroom + *Sciara* sp.+ NSKE + *S. carpocapsae* (1 x 10^2)

T8= Test mushroom + *Sciara* sp.+ NSKE + *S. carpocapsae* (1 x 10^3)

T9= Test mushroom + *Sciara* sp.+ NSKE + *H. indica* (1 x 10^2)

T10= Test mushroom + *Sciara* sp.+ NSKE + *H. indica* (1 x 10^3)

T11= Test mushroom + *Sciara* sp.+ NSKP+ *S. carpocapsae* (1 x 10^2)

T12= Test mushroom + *Sciara* sp.+ NSKP+ *S. carpocapsae* (1 x 10^3)

T13= Test mushroom + *Sciara* sp. + NSKP+*H. indica* (1 x 10^2)

T14= Test mushroom + *Sciara* sp. + NSKP+*H. indica* (1 x 10^3)

T15= Test mushroom + *Sciara* sp. (Untreated Control)

Following observations were recorded:

i. **Mycelial depletion**

Observations were recorded for the mycelial depletion at 2, 4, 6, 8 and 10 days of insect inoculation and per cent mycelial depletion was calculated.

ii. **Larval count**

Larval count per plate (alive maggots) was recorded at the end of the experiment.

3.8.2. *In vivo* experiment

Two experiments on biomanagement of *Sciara* sp. one each in *A. bisporus* and *P. sajor caju* were laid wherein two Neem products viz. NSKE @ 1.0 per cent on v/w basis, NSKP @ 1.0 per cent on w/w basis and two entomophagous nematodes viz. *S. carpocapsae* and *H. indica* each @ 10,000 and 1, 00,000 infective juveniles (IJs) were tested for their efficacy against the test insect in one kg compost/substrate bags. Bioagents were mixed at the given concentrations separately in each bag. The treatments were given at spawning, casing and both at time of spawning and casing in *A.*

bisporus growing bags. In management experiment on *P. sajor caju,* the bags were treated at spawning, at pin head stages and both at spawning and pin head formation stage. Twenty newly emerged larvae of sciarid fly were inoculated to compost/substrate at the time of their respective treatments. Each treatment was replicated three times. Untreated bags were maintained as control. Data were analyzed through CRD. The treatments for each time inoculation (spawning time, casing time/ pin head initiation time) and experiment were as follows:

Tl = Test mushroom + *Sciara* sp.+ Neem Seed Kernel Extract (1%w/v)

T2 = Test mushroom + *Sciara* sp.+ Neem Seed Kernel powder (1 %w/w)

T3= Test mushroom + *Sciara* sp.+ *S. carpocapsae* (1 x 10^4)

T4= Test mushroom + *Sciara* sp.+ *S. carpocapsae* (1 x 10^5)

T5= Test mushroom + *Sciara* sp.+ *H. indica* (1 x 10^4)

T6= Test mushroom + *Sciara* sp.+ *H. indica* (1 x 10^5)

T7= Test mushroom + *Sciara* sp.+ NSKE + *S. carpocapsae* (1 x 10^4)

T8= Test mushroom + *Sciara* sp.+ NSKE + *S. carpocapsae* (1 x 10^5)

T9= Test mushroom + *Sciara* sp.+ NSKE + *H. indica* (1 x 10^4)

T10= Test mushroom + *Sciara* sp.+ NSKE + *H. indica* (1 x 10^5)

T11= Test mushroom + *Sciara* sp.+ NSKP+ *S. carpocapsae* (1 x 10^4)

T12= Test mushroom + *Sciara* sp.+ NSKP+ *S. carpocapsae* (1 x 10^5)

T13= Test mushroom + *Sciara* sp.+ NSKP+*H. indica* (1 x 10^4)

T14= Test mushroom + *Sciara* sp.+ NSKP+*H. indica* (1 x 10^5)

T15= Test mushroom + *Sciara* sp.

T16= Test mushroom only (Untreated control)

Following observations were recorded:

i. **Mycelial growth/ depletion**

Mycelial growth/ depletion were recorded at casing and 45 days of casing in the experiments on biomanagement of *Sciara* in *A. bisporus* as per the time of treatment and inoculation.

Similarly, in oyster mushroom, the mycelial growth/depletion was recorded at pin head initiation and 45 days of pin head formation as per the inoculation and treatment time.

ii. **Total sporophore yield**

Sporophore yield on day to day basis was recorded for each replication of all the treatments as well as controls from day one of the fruit body appearance to the end of cropping. As fruit bodies in these mushrooms normally appear in succession of distinct flushes at 6-7 days gap, weekly yields were pooled to assess the yield per flush. Data of yield per flush were finally pooled to quantify total sporophore production in various treatments.

iii. **Visual symptoms on sporophore**

Visual symptoms, if any, especially on fruiting bodies emerging from the untreated highly infested cropping bags were monitored.

CHAPTER-4

EXPERIMENTAL RESULTS

The survey studies conducted to work out the incidence of insect fauna associated with two commercially grown mushrooms viz. *Agaricus bisporus* (Lange) Imbach (white button mushroom) and *Pleurotus sajor caju* Singer (oyster mushroom) revealed the abundance of flies and several genera of beetles belonging to different families of their respective orders. The flies were *Sciara* sp. (Diptera: Sciaridae) and *Megaselia* sp. Rhondani (Diptera: Phoridae). Beetles recorded in substantial numbers from one or more locations were *Cyllodes indicus* Grouvelle (Coleoptera: Nitidulidae), *Scaphisoma nigrofasciatum* Pic. (Coleoptera: Scaphidiidae), *Spondotriplax pallidipes* Arrow (Coleoptera: Erotylidae) (Pleasing Fungus Beetles) and *Staphylinus* sp. (Coleoptera: Staphylinidae). Two genera of small, soft bodied springtails belonging to Class and Order Collembola viz., *Achorutes armatus* Nic. (Poduridae) and *Lepidocyrtus cyaneus* Tullberg (Entomobryidae) were also observed congregating the dark and damp areas of cropping rooms/ beds/ bags in many units. Presence of mites was also observed at most of the locations. Since the present studies were confined to dipteran pests, detailed investigations on seasonal abundance, phoresy, biology, morphometrics and damaging potential of the most prevalent fly i.e. *Sciara* sp. were undertaken on both the test mushrooms. Experiments to control the test insect were conducted by using eco/ crop friendly bioagents like Neem seed kernel extract, Neem seed kernel powder and two entomophagous nematodes viz., *Steinernema carpocapsae* Weiser and *Heterorhabditis indica* Poinar *et al*.

4.1 Faunistic survey

Compost/substrate and/or casing samples were collected from the cropping bags of various distantly located mushroom units. Sporophore samples were also picked up from wherever visible insect fauna was observed damaging the fruiting bodies. In all, 23 locations of all the nine mushroom growing districts viz., Bilaspur, Hamirpur, Kangra, Kullu, Mandi, Shimla, Sirmaur, Solan and Una were covered during the survey. Mushroom cultivation was not operational in the districts of Chamba, Kinnaur and Lahaul & Spiti. Total 87 samples comprising of growing medium (compost/substrate), from cropping bags, top dressing i.e. casing soil, sporophores and platform soil were collected and analyzed for various pests including insects, collembola and mites. The samples were brought to the laboratory and analyzed under microscope/ hand lens and with naked eye. The faunistic revelations regarding the insect pests in two test mushrooms have been divulged in Figures 1- 7 and Table 1a and 1b.

4.1.1 Insect fauna of *Agaricus bisporus*

Faunistic incidence of insect pests in *A. bisporus* has been referred in Table 1a. The insects belonging to two orders viz., Diptera and Coleoptera were significant by their presence/ abundance in the mushroom units cultivating white button mushrooms. Order Diptera was represented by the flies belonging to two families viz., Sciaridae and Phoridae. Three genera of beetles belonging to different families of Order Coleoptera were noticed in the cropping bags/ rooms. Besides, springtails (Class and Order Collembola) and mites (Class Arachnida) were found to be of common occurrence in the units under survey.

Among Dipterans, Sciarid flies were found to be most widely distributed as they prevailed in 71 out of the total of 87 samples collected from 22 units located in nine districts of the state. No pest infestation was observed in the purposely built commercial farm of Paonta Sahib (District Sirmaur) due to proper hygiene and scientific methods of cultivation adopted there. The flies belonging to genus *Sciara* sp. were found to be prevalent in the cropping beds (Plate 1). These flies were attracted to the aroma of compost and were captured in large numbers on yellow traps laid to monitor the adult population in the units located at Jatoli and Nauni (Distt. Solan) and Maryog (Distt. Sirmaur). While most of the adults hovered over the cropping bags, their maggots were mainly found in compost as well as casing medium. Larval forms were also spotted in large numbers inside the sporophores when they were cut open. Larval population of sciarids ranged between 3-85/250 cc sample in different units depending upon the prophylactic measures adopted against these flies by the growers. Lowest count of three individuals of *Sciara* sp was recorded in Kathiyala (Distt. Kullu) and the highest population of 85.0 were recorded in Sehal (Distt. Kangra). Prevalence of these flies in such high numbers in most of the farms was much above the threshold level of one adult. Interestingly, phorids were observed to be of rare occurrence and were encountered in small numbers in three units only, out of total 23 surveyed. Their count varied from 5-29/250 cc sample; minimum restricted to five in Nauni (Distt. Solan) and maximum of 29 counted in University Farm, Kangra. All the phorids collected during these studies were identified as *Megaselia* sp.

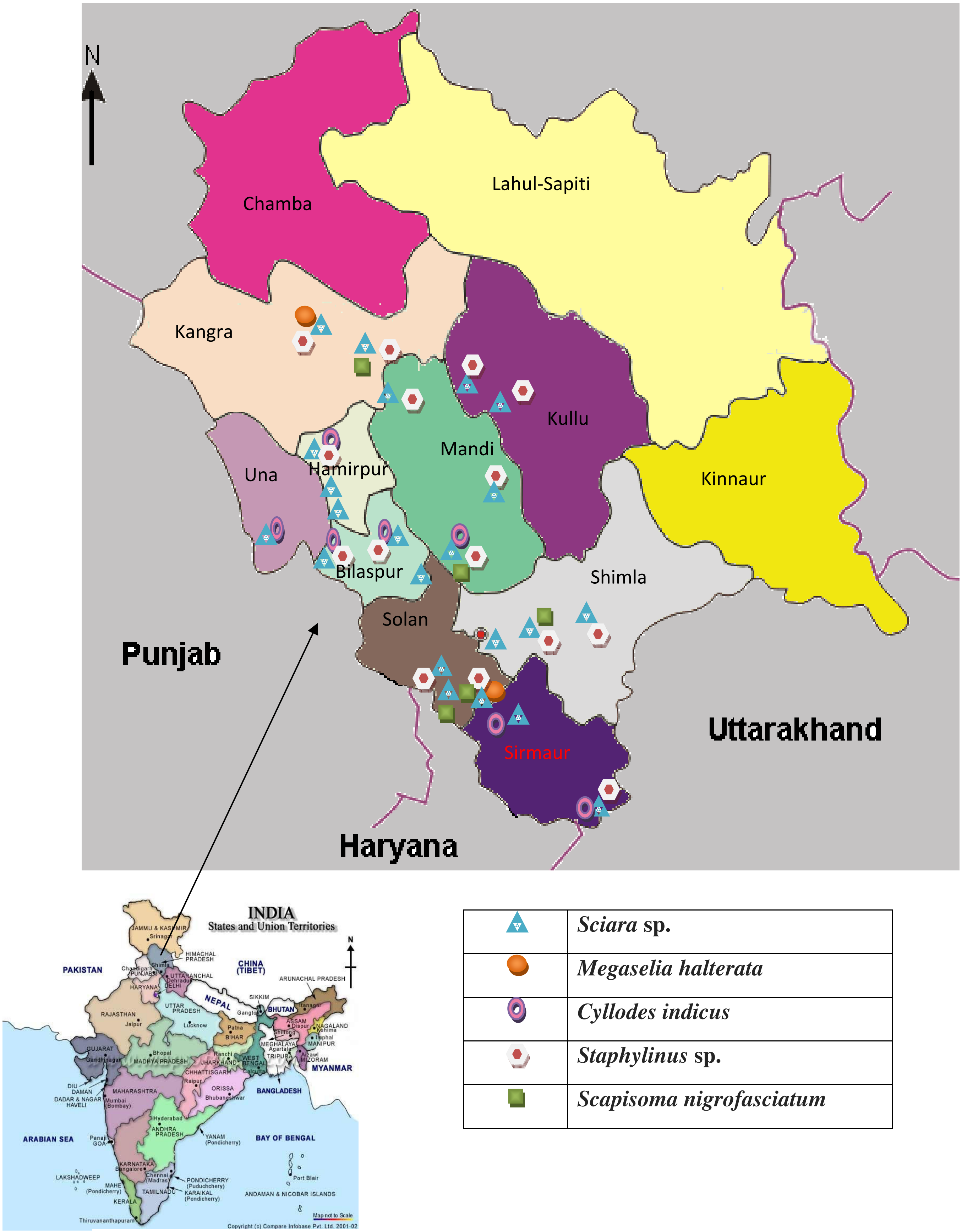


Figure 1. Distribution of insect pests associated with *Agaricus bisporus* in H.P.

Table 1a Insect fauna associated with white button mushroom, *Agaricus bisporus* in Himachal Pradesh

Sr. No	Location	Sample description	DIPTERA						COLEOPTERA					
			Sciarids (*Sciara* sp.)			Phorids (*Megaselia* sp.)			*Cyllodes indicus*		*Staphylinus* sp.		*Scaphisoma nigrofasciatum*	
			Maggots/ 250 cc sample	Pre-pupae/ Pupae per 250cc sample	Adults	Maggots/ 250 cc sample	Pre-pupae/ Pupae per 250cc sample	Adults	Grubs	Adults	Grubs	Adults	Grubs	Adults
Distt. Bilaspur														
1	**Darin**	CB	29	13	NA	-	-	-	11	7	1	2	-	-
2		CB	56	24	NA	-	-	-	-	-	-	-	-	-
3		CB	33	6	NA	-	-	-	-	-	-	-	-	-
4		CA	-	-	NA	-	-	-	-	-	-	-	-	-
5		S	15-	-	1	-	-	-	10	2	-	-	-	-
6	**Samlohal**	CB	28	5	NA	-	-	-	-	-	16	9	-	-
7		CB	35	4	NA	-	-	-	-	-	-	-	-	-
8		CA	10	-	NA	-	-	-	-	-	-	1	-	-
9		S	22	-	-	-	-	-	2	3	-	-	-	-
10	**Kandror**	CB	7	1	NA	-	-	-	-	-	-	-	-	-
11		CB	10	-	NA	-	-	-	-	-	-	-	-	-
12		CA	3	1	NA	-	-	-	-	-	-	-	-	-
13		PS	-	-	NA	-	-	-	-	-	-	-	-	-
14		S	16	-	-	-	-	-	-	-	-	-	-	-
Distt. Hamirpur														
15	**Mansai**	CA	5	-	NA	-	-	-	-	-	-	-	-	-
16		CB	8	7	NA	-	-	-	-	-	-	-	-	-
17		S	4	-	NA	-	-	-	-	-	-	-	-	-
18	**Hareta**	CA	-	-	NA	-	-	-	-	-	-	-	-	-

19		CB	15	9	NA	-	-	-	6	10	-	-	-	-
20		CB	12	10	NA	-	-	-	-	-	-	-	-	-
21	**Kuthera**	CA	6	3	NA	-	-	-	16	22	1	3	-	-
22		CA	-	-	NA	-	-	-	-	-	-	1	-	-
23		CB	13	7	NA	-	-	-	-	-	-	-	-	-
24		S	18	12	1	-	-	-	2	5	2	2	-	-
Distt. Kangra														
25	**Univ. farm**	CB	12	9	NA	29	7	3	-	-	-	-	-	-
26		CA	5	-	NA	-	-	-	-	-	7	7	-	-
27		CB	6	3	NA	6	3	5	-	-	-	-	-	-
28		PS	15	11	NA	12	7	4	-	-	-	-	-	-
29		S	11	4	NA	7	2	3	-	-	2	3	-	-
30		S	16	12	NA	10	5	2	-	-	19	3	-	-
31	**Sehal**	CA	3	-	NA	-	-	-	-	-	-	-	-	-
32		CB	19	4	NA	-	-	-	-	-	3	4	4	1
33		CB	85	14	NA	-	-	-	-	-	2	2	-	-
Distt. Kullu														
34	Kathiyala	CB	2	-	NA	-	-	-	-	-	-	-	-	-
35		CA	-	-	NA	-	-	-	-	-	-	-	-	-
36		CB	3	1	NA	-	-	-	-	-	-	-	-	-
37	Rampur 17 Mile	CA	-	-	NA	-	-	-	-	-	-	-	-	-
38		CB	3	-	NA	-	-	-	-	-	-	-	-	-
39		S	-	-	NA	-	-	-	-	-	2	3	-	-
Distt. Mandi														
40	Dhangiyara	CA	5	3	NA	-	-	-	-	-	1	1	-	-
41		CB	12	6	NA	-	-	-	-	-	1	2	-	-
42		CB	18	8	NA	-	-	-	7	12	-	-	-	-

43		S	10	5	NA	-	-	-	-		1	1	-	-
44	Sohar	CB	13	8	NA	-	-	-	23	18	3	1	2	1
45		CA	8	5	NA	-	-	-	5	4	-	-	-	-
46		CB	10	4	NA	-	-	-	-	-	-	-	-	--
47		S	7	5	NA	-	-	-	2	2				
48	Sandhol	CB	8	5	NA	-	-	-	-	-	-	-	-	-
49		CB	15	6	NA	-	-	-	-	-	1	1	-	-
50		S	10	5	NA	-	-	-	-	-	2	1	-	-
51		S	8	2	NA	-	-	-	-	-	1	2	-	-
Distt. Shimla														
52	**Fagu**	CB	10	3	NA	-	-	-	-	-	1	1	-	-
53		CA	-	-	NA	-	-	-	-	-	-	-	-	-
54		CB	-	-	NA	-	-	-	-	-	-	-	-	-
55		S	8	3	NA	-	-	-	-	-	1	1	-	-
56	**Theog**	CB	12	6	NA	-	-	-	-	-	1	2	-	-
57		CB	17	5	NA	-	-	-	-	-	1	1	-	-
58		CA	6	3	NA	-	-	-	-	-	-	-	-	-
59		S	9	4	NA	-	-	-	-	-	2	2	1	2
60	**Shimla**	CB	12	4	NA	-	-	-	-	-	-	-	-	-
61		CA	5	1	NA	-	-	-	-	-	-	-	-	-
62		S	18	2	NA	-	-	-	-	-	-	-	-	-
Distt. Sirmaur														
63	**Maryog**	CB	27	12	NA	-	-	-	5	3	-	-	-	-
64		CA	10	6	NA	-	-	-	-	1	-	-	-	-
65		CB	12	4	NA	-	-	-	1	2	-	-	-	-
66		CB	28	2	417	-	-	-	-	-	-	-	-	-
67		S	19	-	2	-	-	-	3	4	-	-	-	-
68	**Dumki**	CA	-	-	NA	-	-	-	-	1	2	1	-	-
69		CB	5	2	NA	-	-	-	-	-	-	-	-	-

70		CB	8	6	NA	-	-	-	1	2	1	1	-	
71	**Paonta Sahib**	CB	-	-	NA	-	-	-	-	-	-	-	-	-
Distt. Solan														
72	Nauni	CB	25	9	516	13	5	5	-	-	1	1	-	-
73		CA	8	2	-	10	7	3	-	-	-	-	-	-
74		CB	12	-	NA	5	1	4	-	-	-	-	-	-
75		CB	6	-	-	9	5	4	-	-	-	-	-	-
76		S	-	-	-	-	-	-	-	-	-	1	1	1
77	Shamlech	CA	5	2	NA	-	-	-	-	-	-	-	-	-
78		CB	15	7	NA	-	-	-	-	-	2	1	-	-
79		S	8	2	NA	-	-	-	-	-	1	1	-	-
80	Jatoli	CB	32	15	810	-	-	-	-	-	-	-	3	4
81		CA	12	5	-	-	-	-	-	-	-	-	-	-
82		CB	19	11	NA	-	-	-	-	-	-	-	7	2
83		S	26	-	4	-	-	-	-	-	-	-	-	-
Distt. Una														
84	**Una**	CB	12	18	NA	-	-	-	7	2	-	-	-	-
85		CA	-	-	NA	-	-	-	-	-	-	-	-	-
86		CB	25	12	NA	-	-	-	-	-	-	-	-	-
87		S	-	-	NA									

CB: Cropping Bag CA: Casing Soil S: Spawned bags PS: Platform soil - Not present

The beetles encountered in the cropping bags *A. bisporus* were identified as *Cyllodes indicus*, *Staphylinus* sp. and *Scaphisoma nigrofasciatum*. The most widely distributed among these was *Staphylinus* sp. which infested 30 out of total 87 samples analyzed. While low population of this beetle was recorded in most of the samples, its incidence was higher in Samlohal (Distt. Bilaspur) and University farm (Distt. Kangra) where 16 grubs and nine adults and 19 grubs and three adults per 250 cc sample, respectively were recorded. This was followed by *Cyllodes indicus* that occurred in 17 samples collected from mushroom units located at Darin, Samlohal, Hareta, Kutehra, Dhangiara, Sohar, Maryog Dumki and Una. Grubs and adults were prevalent in the compost and casing samples collected from the cropping bags of the referred areas. Highest population of this beetle was observed in Sohar (Distt. Mandi) with 23 grubs and 18 adults per 250 cc sample. *Scaphisoma nigrofasciatum* was of rare occurrence and its grubs as well as adults were seen in small numbers in six samples collected from Sehal, Sohar, Theog, Nauni and Jatoli. All these beetles and their grubs were voracious feeders and fed upon the mycelium leaving it totally devitalized. They also fed upon sporophores and damaged them to the extent that they became unmarketable.

4.1.2 Insect fauna of *Pleurotus sajor caju*

Faunistic incidence for insects associated with oyster mushroom (*P. sajor caju*) was worked out during survey of nine commercial farms cultivating oyster mushroom located in six districts of the state (Table 1b). These farms were located in Darin (Distt. Bilaspur), Kango (Distt. Hamirpur), Gander (Distt. Kangra), Larenkelo (Distt. Kullu), Maryog, Dumki (Distt. Sirmaur), Shamlech, Nauni and Oachghat (Distt. Solan). In all, 18 substrate samples were collected from the cropping bags. Analysis of the samples exhibited the abundance of sciarid flies in all the samples collected from different areas despite their far away distances from each other suggesting wide distribution of this insect in oyster mushrooms. Larval population in the range of 5-94/ 250 cc substrate was assessed in these samples. Substantial pupal and adult populations were also recorded in all the samples. Thousands of adult flies were found stuck to the yellow traps laid at the farms of Maryog and Nauni for this purpose. This level of inoculum was much above the threshold level of one adult, thus indicating these flies to be the major problem on *P. sajor caju.* Interestingly, phorid fly of *Megaselia* sp. was encountered only at university farm of Nauni (Solan).

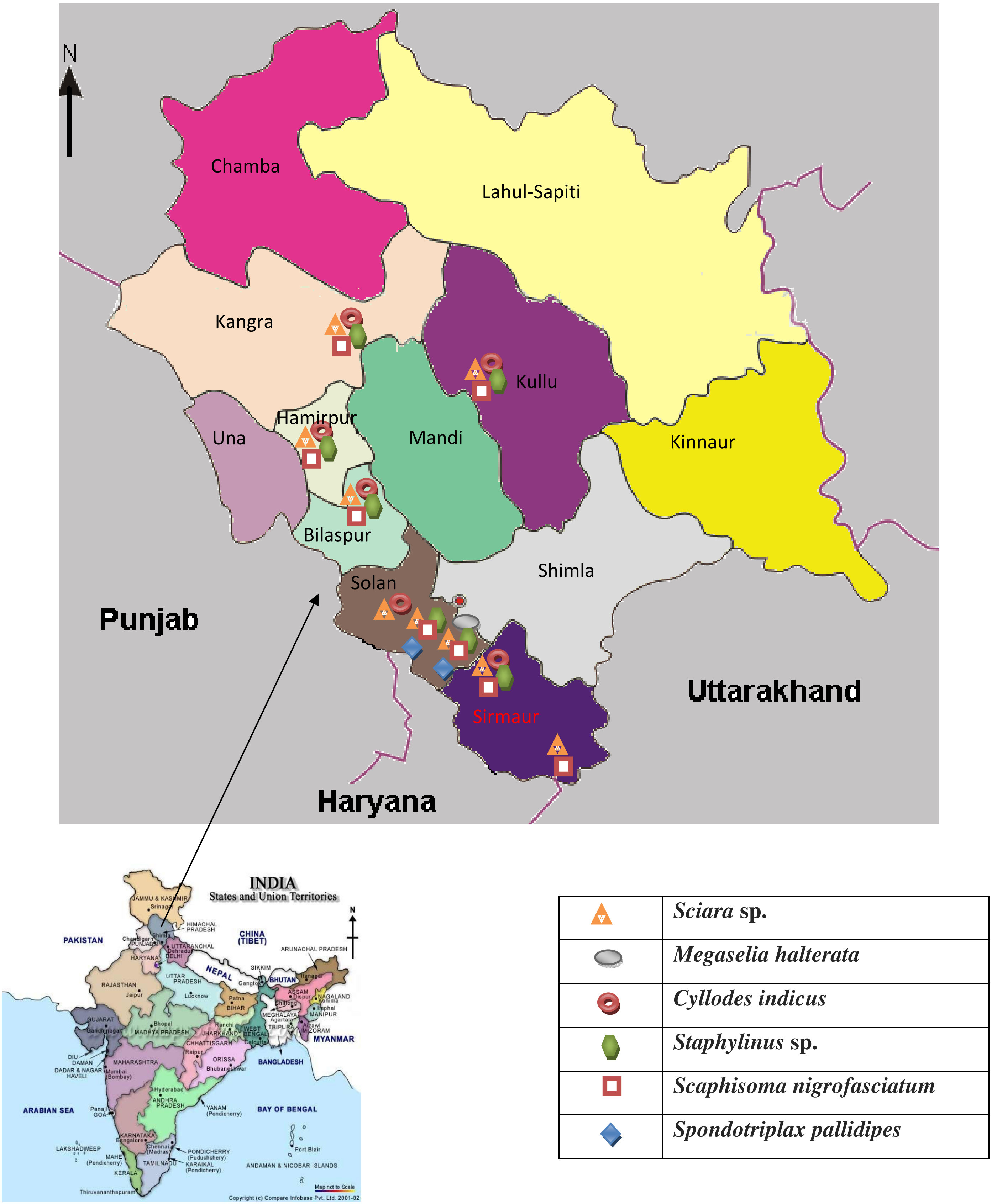


Figure 2. Distribution of insect pests associated with *Pleurotus sajor caju* in H.P.

Table 1b Insect fauna associated with oyster mushroom, *Pleurotus sajor caju* in Himachal Pradesh

Sr. No	Location	DIPTERA						COLEOPTERA							
		Sciarids (*Sciara* sp.)			Phorids (*Megaselia* sp.)			*Cyllodes indicus*		*Staphylinus* sp.		*Scaphisoma nigrofasciatum*		*Spondotriplax pallidipes*	
		Maggots/ 250 cc sample	Pre-pupae/ Pupae per 250cc sample	Adults	Maggots/ 250 cc sample	Pre-pupae/ Pupae per 250cc sample	Adults	Grubs	Adults	Grubs	Adults	Grubs	Adults	Grubs	Adults
	Distt. Bilaspur														
1	Darin	34	6	3	-	-	-	25	10	6	4	5	8	-	-
2		18	7	5	-	-	-	12	4	-	-	1	2	-	-
	Distt. Hamirpur														
3	Kango	8	2	5	-	-	-	-	-	18	16	-	-	-	-
4		10	3	4	-	-	-	3	5	-	-	8	12	-	-
	Distt. Kangra														
5	Gander	47	13	15	-	-	-	26	13	1	2	2	2	-	-
	Distt. Kullu														
6	Larenkelo	8	3	4	-	-	-	5	8	-	3	2	7	-	-
7		6	4	2	-	-	-	-	-	1	2	1	1	-	-
	Distt. Sirmaur														
8	Maryog	27	22	815	-	-	-	17	10	-	-	11	32	-	-
9		18	8	5	-	-	-	1	2	1	1	-	-	-	-
10	Dumki	35	9	12	-	-	-	-	-	-	-	1	2	-	-
11		10	4	3	-	-	-	-	-	-	-	1	1	-	-
	Distt. Solan														
12	Shamlech	5	2	22	-	-	-	15	13	-	-	-	-	-	-
13		12	5	6	-	-	-	-	-	-	-	-	-	-	-
14	Nauni	94	27	1000	12	5	4	28	17	3	5	-	-	-	-
15		35	12	4	3	-	1	12	22	-	-	25	15	48	12
16		24	13	6	8	2	4	-	-	28	24	2	2	30	8
17	Oachghat	10	5	7	-	-	-	-	-	2	1	3	5	-	-
18		45	25	124	-	-	-	-	-	-	2	3	2	7	2

- Not present

Four genera of beetles viz., *Cyllodes indicus*, *Scaphisoma nigrofasciatum, Staphylinus* sp. and *Spondotriplax pallidipes* were observed. Of these, the most abundant were shining fungus beetles *S. nigrofasciatum* (recorded in 13 out of 18 samples) followed by sap beetles, *C. indicus* (observed in ten out of 18 samples). Rove beetles belonging to *Staphylinus* sp. were present in nine samples. Another beetle, *S. pallidipes* was recorded for the first time from mushrooms. This beetle showed its presence in the mushroom substrate at two close by locations i.e. Oachghat and Nauni (Distt. Solan). Populations comprising of 48 grubs and 12 adults and 30 grubs and 8 adults of this beetle were found in two samples collected from Nauni. Comparatively low count of seven grubs and two adults was recovered from Oachghat. As this insect is recorded from mushrooms for the first time, its range of distribution, population levels and damaging potential on this crop needs to be monitored in near future lest it acquires the status as a pest.

The insect fauna of two mushrooms was generally same barring *Spondotriplax pallidipes* (Coleoptera) which was reported for the first time from oyster mushrooms only.

4.1.3 Frequency of Incidence of insect pests associated with mushrooms

Locality vise frequency of incidence of dipteran and coleopteran insects associated with mushrooms was determined and has been demonstrated in Tables 2a and b.

Among white button mushrooms, the most abundant and widely distributed were sciarid flies that harboured all the traditional farms located in distant localities barring one purposely built scientific farm at Paonta Sahib (Table 2a). The frequency of incidence of these flies varied from 33.3 to 100.0 per cent. Cent per cent incidence of these flies was recorded in distantly located mushroom units of Samlohal, Mansai, University farm, Kangra, Sehal, Dhangiyara, Sohar, Sandhol, Theog, Shimla, Shamlech and Jatoli. Lower level of incidence was observed in Kullu and Una. The samples collected from Kathiyala and Rampur 17 Mile farms of district Kullu showed respective incidence of 66.7 and 33.3 per cent. Only 50.0 per cent of the samples from Una showed infestation of sciarid flies. The only phorid retrieved from mushrooms was *Megaselia* sp. This hump backed fly was not as widely distributed and registered its presence in two farms only. University farms of Kangra and Solan suffered respective infestation of 83.3 and 80.0 per cent.

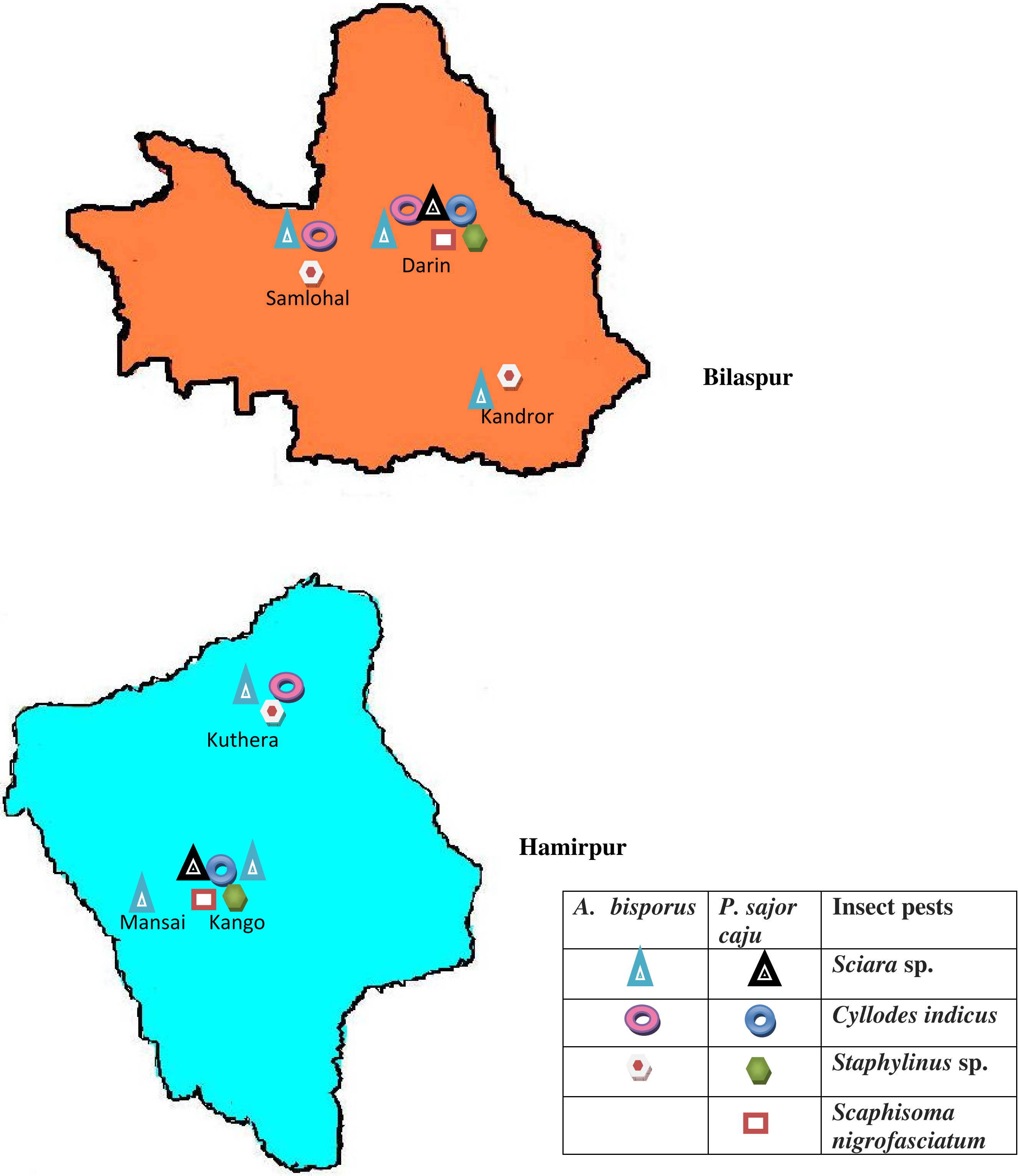


Figure 3: Distribution of insect pests of *Agaricus bisporus* and *Pleurotus sajor caju* in Bilaspur and Hamirpur

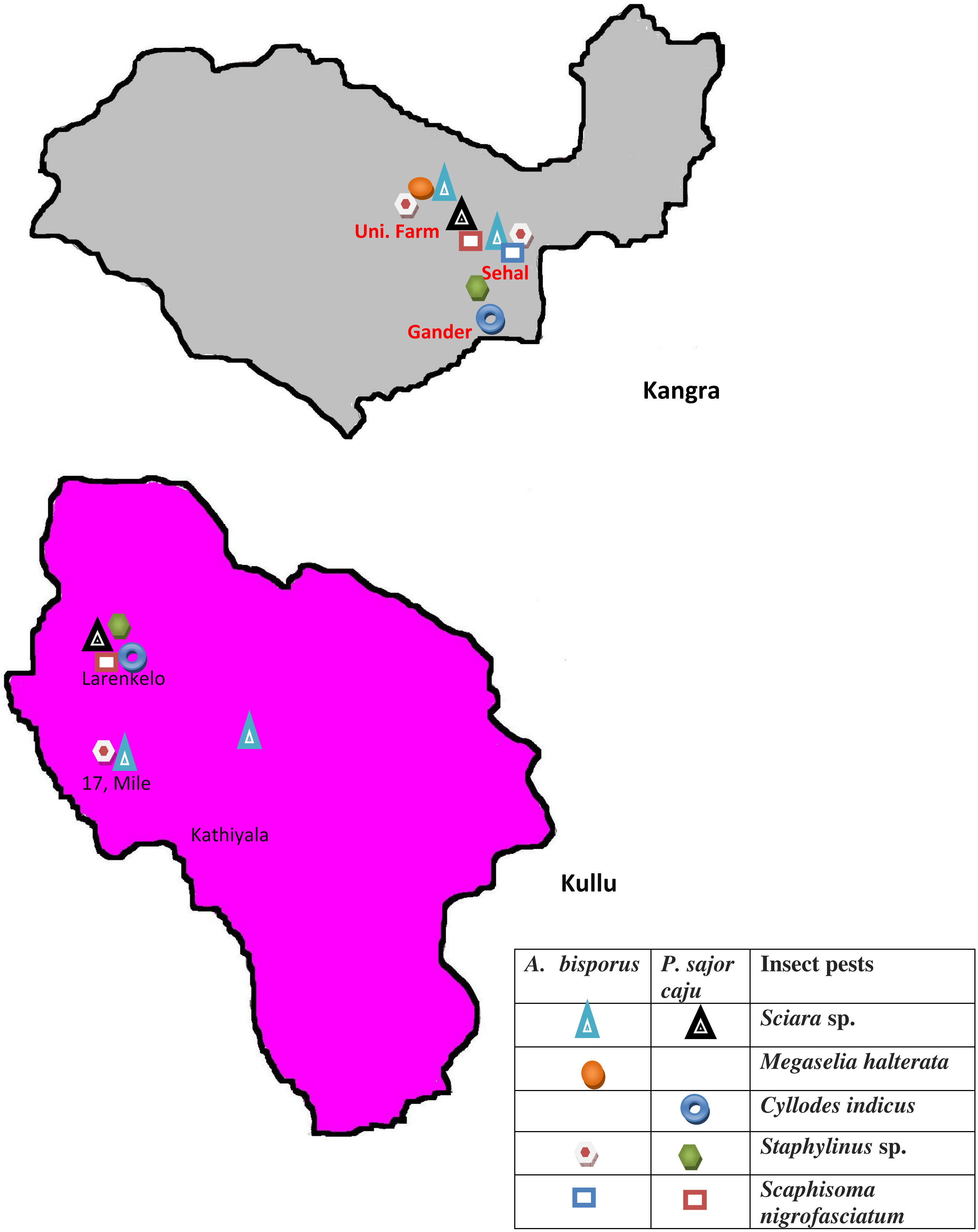


Figure 4: Distribution of insect pests of *Agaricus bisporus* and *Pleurotus sajor caju* in Kangra and Kullu

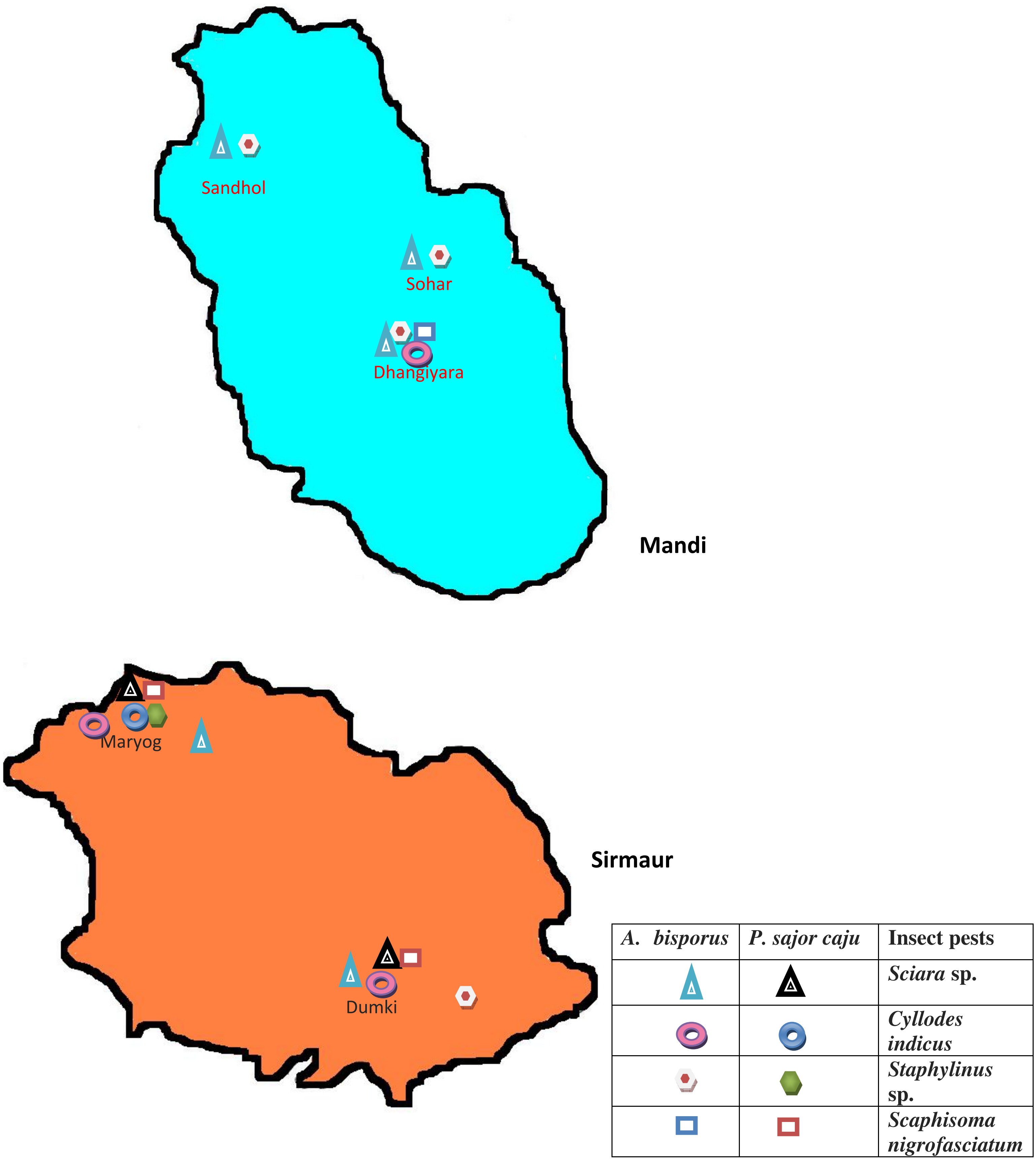
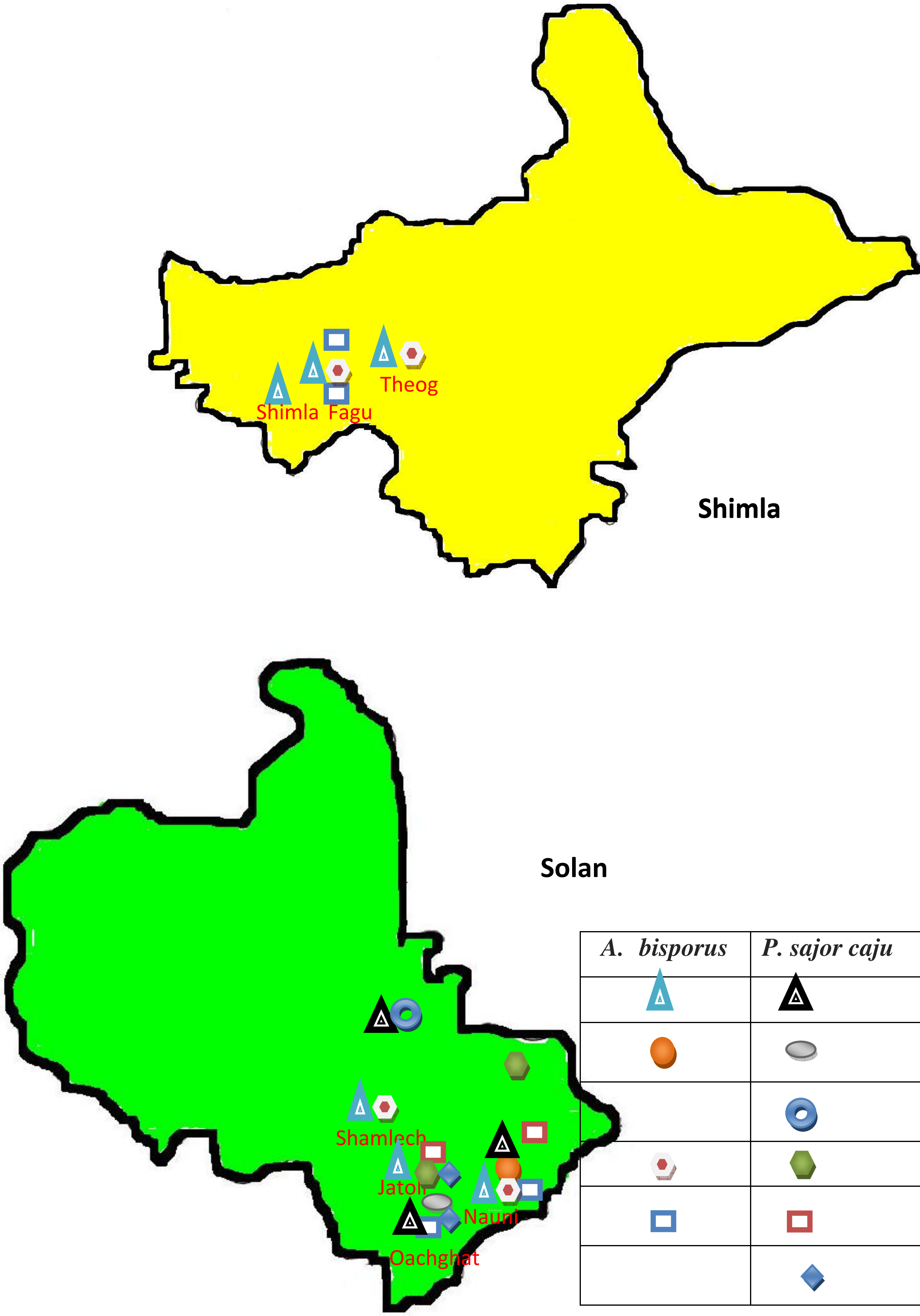


Figure 5: Distribution of insect pests of *Agaricus bisporus* and *Pleurotus sajor caju* in Mandi and Sirmaur



A. bisporus	P. sajor caju	Insect pests
		Sciara sp.
		Megaselia halterata
		Cyllodes indicus
		Staphylinus sp.
		Scaphisoma nigrofasciatum
		Spondotriplax pallidips

Figure 6: Distribution of insect pests of *Agaricus bisporus* and *Pleurotus sajor caju* in Shimla and Solan

Una

A. bisporus	**Insect pests**
	***Sciara* sp.**
	Cyllodes indicus

Figure 7: Distribution of insect pests of *Agaricus bisporus* and *Pleurotus sajor caju* in Una

Table 2a Frequency of incidence of flies and beetles in *A. bisporus* (involving 23 locations in nine districts of Himachal Pradesh)

Location	**Per cent frequency of incidence**				
	Diptera		**Coleoptera**		
	***Sciara* sp.**	***Megaselia* sp.**	***Cyllodes indicus***	***Scaphisoma nigrofasciatum***	***Staphylinus* sp.**
Distt. Bilaspur					
Darin	80.0	0.0	40.0	0.0	20.0
Samlohal	100.0	0.0	25.0	0.0	50.0
Kandror	80.0	0.0	0.0	0.0	0.0
Distt. Hamirpur					
Mansai	100.0	0.0	0.0	0.0	0.0
Hareta	66.7	0.0	0.0	0.0	0.0
Kuthera	75.0	0.0	50.0	0.0	75.0
Distt. Kangra					
Univ. Farm	100.0	83.3	0.0	0.0	50.0
Sehal	100.0	0.0	0.0	33.3	66.7
Distt. Kullu					
Kathiyala	66.7	0.0	0.0	0.0	0.0
Rampur, 17 Mile	33.3	0.0	0.0	0.0	33.3
Distt. Mandi					
Dhangiyara	100.0	0.0	0.0	0.0	66.7
Sohar	100.0	0.0	75.0	25.0	25.0
Sandhol	100.0	0.0	0.0	0.0	75.0
Distt. Shimla					
Fagu	50.0	0.0	0.0	0.0	50.0
Theog	100.0	0.0	0.0	25.0	75.0
Shimla	100.0	0.0	0.0	0.0	0.0
Distt. Sirmaur					
Maryog	100.0	0.0	60.0	0.0	0.0
Dumki	66.7	0.0	33.3	0.0	66.7
Paonta Sahib	0.0	0.0	0.0	0.0	0.0
Distt. Solan					
Nauni	80.0	80.0	0.0	20.0	40.0
Shamlech	100.0	0.0	0.0	0.0	66.7
Jatoli	100.0	0.0	0.0	50.0	0.0
Distt. Una					
Una	50.0	0.0	25.0	0.0	0.0

Beetles were comparatively less prevalent in white button mushroom. Low incidence of three genera of beetles viz., *C. indicus*, *S. nigrofasciatum* and *Staphylinus* sp. was recorded in a few locations. *Staphylinus* sp. was found to be the most prevalent beetle in *A. bisporus* infesting 50.0 per cent of the total samples collected from various localities. Low populations of this beetle were recorded in 14 localities involving eight districts. Whereas highest incidence of 75.0 per cent was observed in the localities of Kuthera, Theog and Sandhol, lowest incidence was assessed in Darin (Distt. Bilaspur) where only 20.0 per cent samples were found infested with *Staphylinus* sp. Though, infestation of *C. indicus* was observed in seven localities, its highest incidence was recorded in Sohar (Distt. Mandi) wherein 75.0 per cent samples were infested. Another beetle, *S. nigrofasciatum* frequented only five locations of four districts viz., Kangra, Mandi, Shimla and Solan. Highest incidence of this beetle was recorded in Jatoli (Distt. Solan) where 50.0 per cent samples were infested followed by Sehal (Distt. Kangra) with 33.3 per cent incidence. Twenty five per cent of the samples collected from Sohar (Distt. Mandi) and Theog (Distt. Shimla) showed incidence of *S. nigrofasciatum*. Minimum per cent incidence was found in University Farm, Nauni, where only one adult and one grub were observed in one out of five analyzed samples(Table2a).

Among beetles, most frequently occurring was Sap beetle, *C. indicus* that infested 55.6 per cent of the total samples collected from various locations. All the oyster mushroom growing units except the farms located at Dumki (Distt. Sirmaur) and Oachghat (Distt. Solan) suffered from its infestation. Cent per cent infestation of this beetle was recorded in the make shift units of Darin (Distt. Bilaspur), Gander (Distt. Kangra) and Maryog (Distt. Sirmaur). Grubs as well as adults of *C. indicus* were found in low to high counts in these units. Fifty per cent samples collected from Kango (Distt. Hamirpur), Larenkelo (Distt. Kullu) and Nauni (Distt. Solan) showed the presence of this beetle. *Staphylinus* sp. was present in seven of the nine localities. It occurred in 100.0 per cent samples collected from Gander, Larenkelo and Oachghat. Fifty per cent samples collected from Darin. Kango and Maryog bore its presence. The beetles, *S. nigrofasciatum* and *S. pallidipes* were of rare occurrence, former being prevalent in Shamlech and Oachghat areas and later in Nauni and Oachghat areas of district Solan. Fifty per cent samples collected from Oachghat and 66.7 per cent samples collected from Shamlech were infested with *S. nigrofasciatum*. *Spondotriplax pallidipes* was recorded from mushrooms for the first time when 50.0 and 66.7 per cent incidence of this beetle was observed in the samples collected from Oachghat and Nauni (Distt. Solan), respectively.

Comparative observations of Tables 2a and 2b demonstrate that though, the fauna of both the test mushrooms was similar, oyster mushroom was more prone to insect attack and harbored higher populations of various insect pests as compared to white button mushroom. Dipteran fly, *B. tritici* was found to be most prevalent in both the mushrooms as it infested cent per cent samples collected from nine distant locations involving six districts. Phorid fly, *Megaselia* sp. did not register its presence in any of the location barring Nauni (Distt. Solan) where the fly showed its prevalence in 100.0 per cent samples. Oyster mushrooms suffered severe infestation of beetles. The predominant among them in *P. sajor caju* was *C. indicus* but in *A. bisporus* was *Staphylinus* sp.

Table 2b Frequency of incidence of flies and beetles on *P. sajor caju* (involving nine locations in six districts of Himachal Pradesh)

Location	**Per cent frequency of incidence**					
	Diptera		**Coleoptera**			
	***Sciara* sp.**	***Megaselia* sp.**	***Cyllodes indicus***	***Scaphisoma nigrofasciatum***	***Staphylinus* sp.**	***Spondotriplax pallidipes***
Distt. Bilaspur						
Darin	100.0	0.0	100.0	0.0	50.0	0.0
Distt. Hamirpur						
Kango	100.0	0.0	50.0	0.0	50.0	0.0
Distt. Kangra						
Gander	100.0	0.0	100.0	0.0	100.0	0.0
Distt. Kullu						
Larenkelo	100.0	0.0	50.0	0.0	100.0	0.0
Distt. Sirmaur						
Maryog	100.0	0.0	100.0	0.0	50.0	0.0
Dumki	100.0	0.0	0.0	0.0	0.0	0.0
Distt. Solan						
Nauni	100.0	100.0	50.0	0.0	0.0	66.7
Shamlech	100.0	0.0	66.7	66.7	66.7	0.0
Oachghat	100.0	0.0	0.0	50.0	100..0	50.0

4.1.4 Collembola and Mite fauna associated with Mushrooms

Perusal of Table 3 indicated the frequent occurrence of soft bodied minute collembolans belonging to two genera viz. *Achorutes armatus* and *Lepidocyrtus cyaneus* in both the test mushrooms, especially where the bags were kept on the ground in dark and damp makeshift rooms. *Achorutes armatus* was found to be more prevalent genus that showed its presence in low to high numbers in 19 out of 27 locations surveyed. Its count in *A. bisporus* ranged from 4 to 44 in different locations. Eleven out of 24 samples registered the prevalence of *A. armatus* in white button mushroom that amounted to 45.8 per cent incidence. Oyster mushroom seemed to be more preferred host as it harbored higher numbers of springtails than *A. bisporus*. Highest population of *A. armatus* was recorded in the oyster mushroom farm located at Larenkelo (Distt. Kullu) where 232 individuals were collected from 250 cc sample analyzed. Frequency of occurrence of this collembolan in *P. sajor caju* was calculated to the tune of 54.5 per cent. *Lepidocyrtus cyaneus* was not as common and prevailed in low numbers in nine out of 27 locations. Five out of total of 24 samples of *A. bisporus* and four out of 11 samples of *P. sajor caju* were found to harbor individuals of *L. cyaneus,* which amounted to respective incidence of 20.8 and 36.4 per cent in *A. bisporus* and *P. sajor caju*.

Besides, mites were observed to be the most frequently occurring arthropods but since their study was not under the purview of this investigation, their identification and counting were not undertaken. However, looking into their prevalence in significant counts in most of the locations, their presence/absence in all the analyzed samples was marked.

4.1.5 Identification of insect and collembola fauna collected from distantly located mushroom farms

The prevalent insect/ Collembola fauna collected separately from distantly located farms growing white button and oyster mushrooms were brought to the laboratory for identification. Temporary/permanent mounts of immature stages were prepared. The specimens were identified with the help of available literature and taxonomic keys. Identification was got confirmed from the expert taxonomists

A . Diptera

Sciarids: *Sciara sp.* (Diptera: Sciaridae) **(Plate 3)**

Egg: Eggs were dirty white in color with a smooth surface and oval shape. They were so minute that not visible to the naked eyes. Their respective length and breadth measured 0.20 to 0.22 mm and 0.14 to 0.16 mm. The fertilized eggs became transparent by the end of incubation period but the unfertile eggs shriveled and turned brown.

Table 3 Collembola and Mite fauna associated with Mushrooms

Location	Mushroom	No. of Springtails per250cc sample		Mites
		Achorutes armatus	*Lepidocyrtus cyaneus*	
Distt. Bilaspur				
Darin	*Agaricus bisporus*	22	-	+
	Pleurotus sajor caju	202	16	+
Samlohal	*Agaricus bisporus*	-	-	-
Kandror	*Agaricus bisporus*	-	-	+
Distt. Hamirpur				
Mansai	*Agaricus bisporus*	-	-	+
Hareta	*Agaricus bisporus*	14	-	-
Kango	*Pleurotus sajor caju*	-	-	-
Kutehra	*Agaricus bisporus*	10	-	+
Distt. Kangra				
Gander	*Pleurotus sajor caju*	118	-	+
Sehal	*Agaricus bisporus*	-	30	+
Univ. Farm	*Agaricus bisporus*	26	-	+
Distt. Kullu				
Kathiyala	*Agaricus bisporus*	-	6	-
Larenkalo	*Pleurotus sajor caju*	232	14	+
Rampur 17 miles	*Agaricus bisporus*	04	-	+
Distt. Mandi				
Dhangiyara	*Agaricus bisporus*	18	-	+
Sohar	*Agaricus bisporus*	10	-	-
Sandhol	*Agaricus bisporus*	28	-	+
Distt. Shimla				
Fagu	*Agaricus bisporus*	-	-	+
Shimla	*Agaricus bisporus*	-	-	+
Theog	*Agaricus bisporus*	-	32	+
Distt. Sirmaur				
Maryog	*Agaricus bisporus*	22	-	+
	Pleurotus sajor caju	112	-	+
Dumki	*Agaricus bisporus*	-	-	+
	Pleurotus sajor caju	20	-	-
Paonta sahib	*Agaricus bisporus*	-	-	-
Distt. Solan				
Jatoli	*Agaricus bisporus*	14	16	+
	Pleurotus sajor caju	66	-	+
Nauni	*Agaricus bisporus*	44	46	+
	Pleurotus sajor caju	-	40	+
Oachghat	*Agaricus bisporus*	35	-	+
	Pleurotus sajor caju	212	46	+

\+ Present - Absent

Maggot: Freshly hatched maggots were cylindrical in shape with body tapering towards the tail end. These maggots possessed a conspicuous shining black head. Newly emerged maggots measured 050-0.64mm in length that reached the maximum of 5.50-5.70 mm by the end of larval period.

Pre pupa and pupa: Maggots turned into non-feeding transformation phase i.e. pre-pupa measuring 2.10-2.76 mm in length and 0.20-0.28 mm in breadth. The prepupa was smaller in size as compared to last larval instar and looked shriveled with appendages articulated to its body. Newly formed pupa was dirty white but its color changed to yellowish brown after 1-2 days of pupation and turned dark grey to black with distinct compound eyes and appendages at maturity. The female pupae differed from male pupae in shape and size, the former being longer and broader than the later. The length of male pupae varied from 1.90-2.20 mm as compared to 2.40-2.70 mm in female pupae.

Adult: Adults were small, delicate, grayish black flies with long thread like filiform antennae having 14 annuli in the flagellum. The female fly was bigger than the male with a broader thorax (0.53±0.04 mm) as compared to that of male (0.40±0.03 mm). Wing of female fly measured 2.13±0.14mm in length and 0.75-0.05mm in breadth, whereas that of male fly was 2.13±0.14mm in length and 0.63-0.03mm in breadth. Wing venation consisted of veins like Costa and subcosta which fused to form thick costal margins in fore wings; radial and radial sector veins were also thick and had a radial cross vein (r) in between. The stem of median vein was faint with thin medial veins forking towards apical margin. The median vein forked into M_{1+2} and M_3 and reached the margin of the wings. In addition to these, Cubital vein forked once and two branches being Cu_1 and Cu_2. Anal veins were absent. A cross vein, radio-medial (r-m) was present but was in line with the distal portion of R_s. In legs, Coxae were contiguous. Abdomen of male was slender and terminated in claspers whereas in females it was swollen and terminated in a pointed ovipositor. Abdomen was distinctly segmented. Length of abdomen in case of female was more as compared to that of male.

Morphometrics of sciarid fly, *Sciara* sp.

Studies on morphometric variations in various stages of *Sciara* sp. infesting *A. bisporus* and *P. sajor caju* have been presented in Table 4a and 4b respectively so as to ascertain the variations if any occurred in the dimensions of various stages of the same species when feeding on different mushroom hosts.

a. Eggs in clusters

b. Larva

c. Pre-pupa

d. Male and Female Pupa

e. Adult Male

f. Adult Female

Plate 3: Life stages of *Sciara* sp. (Diptera : Sciaridae)

A. bisporus

Data presented in Table 4a reveal that egg size ranged from 0.20 to 0.22 mm in length and 0.14 to 0.16mm in breadth. Newly emerged maggots were 0.50 to 0.64 mm in length and breadth varied from 0.06 to 0.10mm. Maggots increased in length and breadth till fourteenth day and average length and breadth of fully matured maggots was recorded 5.58±0.06 mm and 0.50±0.02mm, respectively. Prepupae were reduced in size. Matured female pupae were distinct from male pupae in being broader and longer than their counterpart. Among adults, female flies were larger than males in respect of wing size, breadth of thorax and length of abdomen. Wing size of female fly was 2.13±0.14mm in length and 0.75-0.05mm in breadth, whereas that of male fly was 2.13±0.14mm in length and 0.63-0.03mm in breadth. Dimensions of head capsule shed on day two/three were 0.12±0.01mm and 0.07±0.02mm. Corresponding size of head capsule shed on twelfth/fourteenth day varied from 0.34-0.38mm in length and 0.24-0.28mm in breadth.

P. sajor-caju

Perusal of data presented in Table 4b reveal the morphometric variations in different stages of *Sciara* sp. when developed on fully impregnated mycelium of *P. sajor caju.*

Eggs were microscopic and measured 0.21±0.01mm and 0.16±0.03 mm in length and breadth, respectively. Day to day length of maggots were recorded under the microscope and length of maggots varied from 0.50-0.60 mm at first day to 4.96-5.60 mm on fifteenth day. Average breadth of newly hatched maggot was 0.06±0.01 that increased to 0.42 to 0.44 mm (0.43±0.01mm) on fifteenth day. The length of prepupa reduced as compared to last larval instar and it varied from 2.10 mm to 2.76 mm with averages of 2.50±0.24 mm. the breadth also reduced correspondingly in prepupal stage. Length and breadth of female pupae was greater than male pupae. Length and breadth of female pupae varied from 2.50-2.56 mm and 0.60-0.68 mm, respectively, whereas respective length and breadth of male pupae was recorded 1.90-2.00 and 0.50-0.56 mm. Thorax of female was also broader (0.53±0.04) as compared to that of male (0.40±0.03mm). Adult female had larger wings than males. Length of abdomen in case of female (1.66±0.09 mm) was more as compared to that of male (1.09±0.07 mm). In general, female pupa was bigger than male pupa. Measurements regarding length and breadth of head capsule were also recorded. Length of head capsule measured from 0.10-0.14 mm on 3rd - 4th day to 0.32-0.38 mm head capsules seen on 13th -15th day.

Table 4.a Morphometric variations in different stages of *Sciara* sp. on *A. bisporus*

Immature stages	Measurements (Mean of ten replications)					
	Length (mm)			Breadth (mm)		
	Range	Mean±S.D.	C.V.	Range	Mean±S.D.	C.V.
Egg	0.20-0.22	0.21±0.01	0.05	0.14-0.16	0.15±0.01	0.05
Larva- Day 1	0.50-0.64	0.57±0.05	0.06	0.06-0.10	0.08±0.02	0.19
Day 2	0.76-0.90	0.84±0.06	0.05	0.08-0.10	0.09±0.01	0.14
Day 3	1.00-1.16	1.09±0.05	0.04	0.10-0.12	0.11±0.01	0.09
Day 4	1.14-1.50	1.35±0.09	0.04	0.12-0.16	0.14±0.01	0.08
Day 5	1.60-1.74	1.69±0.06	0.02	0.16-0.22	0.19±0.02	0.06
Day 6	1.84-2.08	1.98±0.08	0.03	0.20-0.24	0.22±0.02	0.06
Day 7	2.10-2.44	2.29±0.10	0.03	0.22-0.24	0.22±0.01	0.06
Day 8	2.70-3.00	2.86±0.10	0.03	0.24-0.30	0.26±0.02	0.04
Day 9	3.16-3.56	3.33±.0.22	0.03	0.28-0.32	0.30±0.01	0.04
Day 10	3.64-4.10	3.92±0.14	0.02	0.30-0.34	0.32±0.01	0.05
Day 11	4.20-4.52	4.36±0.10	0.02	0.34-0.40	0.37±0.02	0.05
Day 12	4.36-4.56	4.45±0.08	0.02	0.36-0.44	0.39±0.03	0.03
Day 13	4.54-5.34	4.98±0.27	0.03	0.36-0.44	0.46±0.01	0.02
Day 14	5.50-5.70	5.58±0.06	0.01	0.46-0.52	0.50±0.02	0.04
Pre-pupa	2.30-2.70	2.51±0.12	0.05	0.22-0.28	0.26±0.02	0.10
Pupa – Male	1.92-2.20	2.03±0.10	0.05	0.50-0.56	0.53±0.02	0.03
Pupa- Female	2.40-2.70	2.58±0.09	0.03	0.56-0.70	0.63±0.05	0.07
Adult- Male						
Wing	1.50-1.70	1.62±0.08	0.05	0.58-0.68	0.63-0.03	0.05
Thorax				0.36-0.46	0.41±0.04	0.09
Length of abdomen	1.04-1.24	1.14±0.06	0.06			
Adult- Female						
Wing	1.96-2.36	2.13±0.14	0.06	0.68-0.80	0.75±0.05	0.06
Thorax				0.50-0.72	0.61±0.06	0.10
Length of abdomen	1.60-1.84	1.70±0.08	0.05			
Head Capsule						
Day 2 & 3	0.10-0.14	0.12±0.01	0.12	0.04-0.10	0.07±0.02	0.24
Day 5 & 6	0.18-0.22	0.20±0.01	0.06	0.14-0.18	0.15±0.01	0.09
Day 8 - 9	0.26-0.30	0.28±0.01	0.05	0.20-0.26	0.23±0.02	0.10
Day 12- 14	0.34-0.38	0.36±0.01	0.04	0.24-0.28	0.25±0.01	0.05

Table 4 b. Morphometric variations in different stages of *Sciara* sp. on *P. sajor caju*

Immature stages	Measurements (Mean of ten replications)					
	Length(mm)			Breadth(mm)		
	Range	Mean±S.D.	C.V.	Range	Mean±S.D.	C.V.
Egg	0.20-0.22	0.21±0.01	0.05	0.14-0.16	0.16±0.03	0.06
Larva- Day 1	0.50-0.60	0.55±0.03	0.09	0.04-0.08	0.06±0.01	0.02
Day 2	0.72-0.84	0.79±0.04	0.07	0.06-0.10	0.08±0.01	0.11
Day 3	1.08-1.16	1.12±0.04	0.04	0.10-0.12	0.11±0.01	0.09
Day 4	1.28-1.44	1.36±0.06	0.07	0.12-0.14	0.13±0.01	0.09
Day 5	1.68-1.80	1.73±0.04	0.04	0.16-0.18	0.17±0.01	0.09
Day 6	1.96-2.10	2.04±0.05	0.04	0.20-0.24	0.22±0.01	0.08
Day 7	2.24-2.50	2.38±0.07	0.05	0.22-0.24	0.23±0.01	0.06
Day 8	2.56-2.76	2.65±0.07	0.04	0.24-0.26	0.25±0.01	0.08
Day 9	3.10-3.30	3.21±.0.07	0.04	0.26-0.28	0.27±0.01	0.04
Day 10	3.60-3.80	3.71±0.07	0.04	0.28-0.30	0.29±0.01	0.0.3
Day 11	3.96-4.08	4.03±0.04	0.01	0.30-0.34	0.31±0.04	0.03
Day 12	4.10-4.30	4.22±0.07	0.02	0.34-0.36	0.32±0.02	0.05
Day 13	4.38-4.60	4.49±0.08	0.02	0.36-0.40	0.37±0.02	0.02
Day14	4.62-4.88	4.74±0.013	0.03	0.40-0.42	0.41±0.01	0.02
Day 15	4.96-5.60	5.32 ± 0.19	0.04	0.42-0.44	0.43±0.01	0.03
Pre-pupa	2.10-2.76	2.50±0.24	0.10	0.20-0.24	0.22±0.01	0.07
Pupa – Male	1.90-2.00	1.94±0.03	0.02	0.50-0.56	0.54±0.02	0.04
Pupa- Female	2.50-2.56	2.53±0.03	0.01	0.60-0.68	0.65±0.02	0.04
Adult- Male						
Wing	1.50-1.64	1.59 ±0.04	0.03	0.60-0.64	0.62-0.02	0.03
Thorax				0.34-0.44	0.40±0.03	0.08
Length of abdomen	0.96-1.16	1.09±0.07	0.07			
Adult- Female						
Wing	1.90-2.24	2.08±0.12	0.06	0.70-0.82	0.76±0.04	0.05
Thorax				0.48-0.56	0.53±0.04	0.07
Length of abdomen	1.52-1.84	1.66±0.09	0.06			
Head Capsule						
Day 3 - 4	0.10-0.14	0.12±0.02	0.13	0.04-0.14	0.07±0.02	0.30
Day 6- 7	0.18-0.22	0.20±0.02	0.09	0.14-0.18	0.16±0.02	0.10
Day 8 - 11	0.24-0.30	0.26±0.02	0.09	0.20-0.26	0.23±0.02	0.09
Day 13- 15	0.32-0.38	0.35±0.02	0.06	0.24-0.30	0.28±0.02	0.07

Phorids: *Megaselia* sp. (Diptera: Phoridae) (Plate 4)

The common names viz., 'humpbacked fly', 'scuttle fly' given to the members of family Phoridae signified their typical structure/ behavior like jerky, short bursts of running done by adult fly. The life cycle consisted of four stages including egg, maggot, pupa, and adult. The flies were attracted to the mycelial odor in the spawned compost and entered the cropping rooms through doors, windows and ventilators. The ordinary wire screen meant to prevent the ingress of house flies and mosquitoes did not prevent the entry of these tiny insects. Minute cylindrical eggs were smooth surfaced and of whitish hue. Freshly hatched maggots were transparent with visible white thread like dorsal tracheal vessel. The initial creamish white color of pupa changed to dark brown at maturity. Adults were with rudimentary antennae apparently consisting of a single globular segment, more or less sunken in head cavity. Coxae in the legs were contiguous with each other and hind femora were robust and laterally flattened. The adult males emerged earlier than the females by two days. Male flies were darker as compared to females.

B. Coleoptera

***Staphylinus* sp. (Coleoptera : Staphylinidae) (Plate 5.a)**

Commonly referred as rove beetles, these were slender, elongated, black insects characterized by the very short elytra rounded at the apex. Adults were with distally enlarged moniliform antennae that arose on front or sides of head nearer to eyes. The mandibles were very long, slender and sharp and usually crossed in front of the head. Terminal segment of maxillary palpi were not greatly enlarged. The hind wings were membranous, well developed and when at rest, were folded under the short elytra. Abdomen was exposed beyond elytra and six to eight sterna were visible. These beetles were swift runners and frequently raised the tip of their abdomen much like scorpions while running.

***Scaphisoma nigrofasciatum* Pic. (Coleoptera: Scaphidiidae) (Plate 5.b)**

These shiny convex beetles were pointed at both the ends. Head bore filiform antennae. The elytra were short and truncate at apex leaving only one or two abdominal sterna exposed. Hind wings were folded under the short elytra at rest. All tarsi were distinctly five segmented. Last abdominal segment was pointed, shiny and oval.

a. Larva b. Pupa c. Adult

Plate 4: Developmental stages of *Megaselia* sp.

Plate 5.a Adult of *Staphylinus* sp.
(Coleoptera : Staphylinidae)

Plate 5. b Adult of *Scaphisoma nigrofasciatum*
(Coleoptera : Scaphidiidae)

Cyllodes indicus **Grouvelle (Coleoptera: Nitidulidae) (Plate 6)**

These were small, broadly oval shaped sap beetles with shining black elytra cut off squarely at the apex exposing one or two apical abdominal segments. Elytra usually had two orange spots. The antennae were clubbed and three segmented. Front coxae transverse with a trochantin. When disturbed, these beetles drew in their legs and antennae and became motionless. The appendages fit so snugly in shallow grooves on the ventral side of the body that it was often difficult to observe them, even with considerable magnification.

Spondotriplax pallidipes **Arrow (Coleoptera: Erotylidae) (Plate 7)**

Commonly known as 'Pleasing Fungus Beetles', these insects were recorded for the first time from mushrooms in India. These were medium sized, oval, shiny beetles with three to four segmented clubbed antennae and smooth elytra. Front coxae were globose without trochantin. First segment of hind tarsi was as long as second or even longer. First three abdominal sterna were not fused together, tarsi were distinctly five segmented and had small tarsal claws.

C. Collembola

Achorutes armatus **Nic. (Collembola : Poduridae)**

These were minute to small, soft bodied springtails, often with rudimentary ocelli. Adults were silver grey in color with rounded head having two black ocellar fields on it. Antennae were four segmented; third and fourth segments being ovoid in shape and the terminal segment being larger than the rest. Thorax was tubular and elongated. Abdomen was six segmented indicated by external sutures. The minute hairs covered the entire body. Their distinct feature was the absence of springing organ (furcula). These springtails crawled very fast but on touching/disturbing, they jumped by folding the body in 'U' shape and stretching quickly that resulted in instant jump or bounce. Legs were short and hairy with a pair of claws. Cerci were absent.

Lepidocyrtus cyaneus **Tulb. (Collembola : Entomorbryidae)**

These were minute, soft bodied brown springtails with rudimentary ocelli and chewing or piercing mouthparts. The prothorax was reduced, usually not visible from above and with bristles dorsally. Abdomen was six segmented with a sucker like ventral tube (collophore), a retaining hook (tenaculum) and a furcula (forked jumping organ) on segments 1, 3, and 4, respectively. At rest, the furcula folded forward under the abdomen and was held in place by a clasp like structure called tenaculum. They jumped by extending the furcula ventrally and posteriorly. Cerci were absent.

a. Grub

b. Newly emerged adult

c. Mature Adult

Plate 6: Different stages of *Cyllodes indicus* (Coleoptera: Nitidulidae)

a. Grub

b. Adult

Plate 7: Different stages of *Spondotriplax pallidips* (Coleoptera: Erotylidae)

4.1.6 Nature of damage of dipterans associated with mushrooms

A. Sciarids

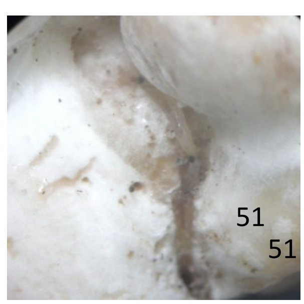
The Sciarid fly (maggot) caused minor to major crop losses intermittently in most of the makeshift mushroom units depending upon their inoculum, time of infestation and the environmental conditions. The maggots preferably attacked and fed upon the thickened junctions of mycelial network. During feeding as they moved from one cell to another, tunneling of the mycelial mat occurred that restrained the uniform impregnation of the mycelium in the bags. The most serious damage occurred on the developing primordia or pin heads wherein mycelial attachments were severed by the maggot thereby causing browning of the newly emerging pinheads. These brown pin heads turned leathery and never developed into buttons/ sporocarps. In *A. bisporus*, when the compost was cased, the maggots moved to casing and preferably pupated there. In *P. sajor caju*, the preferred site for pupation was about 2-2.5 cm deep from the substrate surface but occasionally pupae were also observed on the surface of substrate. Pupae were non feeding/non damaging. The infested pinheads/sporocarps turned hollow and spongy. The maggots also entered the stipe of matured sporophores from its base. Tunneling occurred due to movement of sciarid maggot in the sporophores **(Plate 8 and 9)**.

B. Phorids

The observations were recorded in *P. sajor caju* where the maggots hatching from the eggs laid on mushroom gills directly bore into the mushroom cap whereas those hatching from the eggs laid in spawned compost/ casing material tunneled through the bottom of the mushroom stalk. Maggots fed gregariously and moved upwards forming tunnels till their maturity. The attacked mushrooms bent towards the tunneled stalk and the color of infested mushroom changed to yellowish brown from white. Pin head formation was greatly hampered when the maggot attacked the emerging primordia.

4.2. Biology of sciarid fly (*Sciara* sp.)

The observations regarding different stages of the life cycle and their duration in *Sciara* sp. insect were recorded from egg laying to the death of adults. Biology of *Sciara* sp. was studied on two mushroom hosts' i.e. on *A. bisporus* and *P. sajor caju*. The data recorded have been placed in Table 5a and 5b.



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a. Browning of sporocarps b. Tunneling of pileus c. Tunneling in stipe

d: Drying and browning of emerging pin heads in crop infested with *Sciara* sp.

Plate 8: Visual symptoms produced by *Sciara* sp. in *Agaricus bisporus*

a. Browning of sporocarps b & c. Tunneling by *Sciara* maggots feeding on the sporocarps

d. Bags of oyster mushroom infested with *Sciara* sp.

Plate 9: Visual symptoms produced by *Sciara* sp. in *Pleurotus sajor caju*

4.2.1 Biology of sciarid fly, *Sciara* sp. on *Agaricus bisporus*

The developmental stages of *Sciara* sp. were studied on the mycelium of *A. bisporus* maintained at 23±1^{0}C under laboratory conditions. The relevant information has been presented in Table 5a.

Table 5a Biological parameters of sciarid fly, *Sciara* sp. on white button mushroom, *A. bisporus*

Biological parameters	Range	Mean ± S.D.
Fecundity (eggs/female)	27-56	42.3±8.3
Incubation Period(Days)	2-3	2.5±0.5
Per cent hatchability	81.1-96.3	89.96±4.8
First instar (days)	2-3	2.33±0.4
Second instar (days)	2-3	2.53±0.5
Third instar (days)	2-3	2.40±0.5
Fourth instar (days)	2-4	2.53±0.6
Total Maggot Period (days)	8-13	9.80±1.7
Pre-pupal period (days)	1-3	1.60±0.6
Pupal Period (days)	2-3	2.27±0.4
Total development period(days)	11-19	13.67±2.4
Pupal survival rate (%)	66.7-93.3	82.00±8.9
Pre oviposition period (hrs.)	15-26	21.00±3.4
Mating period (min.)	4-8	5.67±1.2
Post oviposition period (hrs.)	5-8	6.4±1.02
Adult longevity (days) - Female	5-7	6.00±0.7
Adult longevity (days) – Male	4-6	5.17±0.8

Eggs were laid on the mushroom mycelium and were oval, smooth, whitish and minute, not visible to naked eye. An average female laid 27-56 eggs either singly or in clusters of 8 to 12. The average incubation period was 2.5±0.5 days. The mean egg viability was 89.9±4.8 per cent and varied from 81.1-96.3 per cent. The infertile eggs were slightly shriveled and transparent and after 1-2 days they turned brown. Freshly hatched maggot was distinct with shining black head, and was clearly visible with naked eyes. Early instar maggots made the tunnels in mycelium and fed upon mycelium voraciously. The maggot duration ranged from 8 to 13 days with an average of 9.8±1.7 days. The full grown maggots were distinct with black head and visible alimentary canal.

The maggots thrived on the mycelium. There was a prepupal phase of 1 to 3 days with an average of 1.6±0.6 days when maggots stopped feeding, became sluggish and stopped moving to turn in to the pupae. The new pupae were dirty white in color. Their color changed to yellowish brown after one to two days of pupation and the matured pupae were deep grey in color. The compound eyes and appendages became distinctly apparent in pupal phase. In general, male pupae had broader and shorter abdomen than that of females. Pupal period varied from 2 to 3 days with an average of 2.3±0.4. The average per cent survival rate of pupae was 82.0±8.9 (66.7 to 93.3 per cent).

The adult flies were grayish black with elongated abdomen, long legs and wings. The adults preferred moist and dark places of the growing chambers. There were fourteen annuli in the flagellum of the antennae. Adult females lived longer than males. While females survived for five to seven days longevity of males was of four to six days. Copulation was recorded under the captivity condition for the first time under laboratory conditions **(Plate 10)**. They were observed end-to-end "in copula" position on the sides of Petri plates having fully impregnated mycelium as well as on the mycelium. Copula was broken once or twice before actual mating. During this period, while female remained stagnant male made jerky movements around her. Actual mating occurred thereafter. Mating period ranged from four to eight minutes (5.7±1.2 minutes). Once separated, the male made swift movements but female remained stagnant at one place. They did not mate again even when kept together in captivity. Average pre-oviposition period was 21.00±3.39 hours (15-26 hours). The females survived barely for five to eight hours after oviposition (6.4±1.02 hours). Developmental period from egg to adult emergence varied from 13.67 days (13.67±2.44 days).

4.2.2 Biology of sciarid fly, *Sciara* sp. on *Pleurotus sajor caju*

Biology of *Sciara* sp. was studied for the first time on *P. sajor caju* mycelium grown at 26±1^{o}C under laboratory conditions. The information perceived has been placed in Table 5b.

Females laid eggs either singly or in clusters of eight to eleven. The average fecundity was recorded to be 45.9±8.2 eggs (29-58 eggs/female). About 90.2 per cent of the eggs laid were viable (82.8-94.8 per cent). The first instar larva hatched from the egg at three to four days. Four larval instars were differentiated on the basis of the head capsules. Total larval period ranged from 11 to 15 days (11.8± 4.5 days). The prepupal phase remained for two to three days which eventually converted into exarate pupae. The pupal period on an average was of 2.5±0.5 days. The pupal survival rate varied from 66.8 to 93.3 per cent with an average of 78.0±10.8 per cent. The developmental period from egg to adult emergence varied from 14-21 days with average of 16.7±2.8 days.

Developmental period of the test insect delayed by three days in *P. sajor caju* as compared to the time taken in *A. bisporus*. The most probable cause could be the variation in temperature requirement of two test mushrooms as humidity maintained was appreciably similar in both the mushrooms. The insect had an average developmental phase of 13.67 days in white button mushroom being grown at 23.0±1.0^{o}C as compared to 16.70 days in oyster mushroom grown at 26±1^{o}C.

Table 5 b Biological parameters of sciarid fly, *Sciara* sp. on *P. sajor-caju*

Biological parameters	Range	Mean ± S.D.
Fecundity (eggs/female)	29-58	45.9±8.2
Incubation Period(Days)	3-4	3.1±0.5
Per cent hatchability	82.8-94.8	90.2±4.5
First instar (Days)	3-4	3.1±0.5
Second instar (Days)	2-3	2.7±0.5
Third instar (Days)	3-4	3.2±0.6
Fourth instar (Days)	3-4	3.0±0.4
Total Larval Period (days)	11-15	11.8±4.5
Pre-pupal period (days)	2-3	2.5±1.1
Pupal Period (days)	2-3	2.45±0.5
Total development period(days)	14-21	16.7±2.8
Pupal Survival Rate (%)	66.7-93.3	78.00±10.8
Pre oviposition period (hrs.)	16-29	23.00±4.4
Mating period (mts.)	4-8	5.67±1.0
Post oviposition period (hrs.)	4-8	5.42±1.2
Adult longevity (days) –Male	4-6	4.63±0.7
Adult longevity (days) –Female	5-7	5.78±0.8

Average longevity of female and male fly was five to seven days and four to six days, respectively. The mating period ranged from four to eight minutes with an average of 5.7±1.0 minutes. Pre oviposition period on an average was 23.0 ± 4.4 hours and varied from 16-29 hours. Post oviposition was very small varying from four to eight hours with an average of 5.4 ± 1.2 hours.

4.2.3 Sex ratio of *Sciara* sp. on *Agaricus bisporus* and *Pleurotus sajor caju*

The data evaluated on sex ratio on two different mushroom hosts have been manifested in Table 6. Natural populations of *Sciara* sp. associated with white button mushroom when examined for relative number of males and females exhibited that the number of females was always more than males. Studies conducted on different populations collected from mushroom units of Nauni, Jatoli, Maryog, Sehal and Darin showed the respective sex ratios of 1.5, 1.6, 1.3, 1.2 and 1.3. The overall sex ratio was assessed as 1.41.

Sciara adults collected in a natural way from oyster mushroom farms of four locations viz., Nauni, Oachghat, Maryog, and Dumki reflected similar observations as in populations collected from *A. bisporus*. Overall sex ratio in *P. sajor caju* was worked out to be 1.29.

Table 6 Sex ratio of *Sciara* sp. on *Agaricus bisporus* and *Pleurotus sajor caju*

Host	Location	Population size	Females	Males	Sex ratio
Agaricus bisporus	**Nauni (Distt. Solan)**	42	25	17	1.5
	Jatoli (Distt. Solan)	26	16	10	1.6
	Maryog (Distt. Sirmaur)	14	8	6	1.3
	Sehal (Distt. Kangra)	22	12	10	1.2
	Darin (Distt. Bilaspur)	07	04	03	1.3
Total		111	65	46	1.41
Pleurotus sajor caju	**Nauni (Distt. Solan)**	27	16	11	1.45
	Oachghat (Distt. Solan)	13	07	06	1.17
	Maryog (Distt. Sirmaur)	22	12	10	1.20
	Dumki (Distt. Sirmaur)	09	05	04	1.25
Total		71	40	31	1.29

4.3 Phoretic behavior of nematodes/mites

Nematodes and mites, when present in cropping beds, have a tendency to congregate on surface of the substrate/compost/casing medium. When adult insects emerge or sit on the cropping beds/bags, these tiny organisms often cling to their legs, thorax and abdomen. Such insects with nematodes/mites/other tiny arthropods attached to their bodies act as their carriers and help in their dissemination. Insects collected from mushroom units of seven locations suspected to be infested with nematodes/mites were brought to the laboratory. Five individuals of each genus from various locations were selected and when washed individually in small quantity of water, showed the presence of nematodes/mites/unidentified arthropods on their bodies. Data regarding phoretic behavior has been assembled and placed in Table 7a.

Table 7 a Phoretic behavior of mites and nematodes

Locality	Insect	No. of insects washed	No. of carrier insects	Per cent Phoresy	Clinging of N/M/O	Phoretic intensity per insect
Bilaspur	*Sciara* sp.	5	1	20	M/O	3/2
Kangra	*Sciara* sp.	5	-	-	-	-
	Staphylinus *sp.*	5	2	40	M, M	5, 3
	C. indicus	5	4	80	M/O, N/M, M, N/M	22/1, 4/12, 18, 3/23
	S. nigrofasciatum	5	3	60	N/M, N/M, N	5/3, 4/1, 7
Kullu	*Sciara sp.*	5	1	20	N	5
	Staphylinus sp.	5	2	40	N/M, M/O	7/4, 8/1
Mandi	*Sciara sp.*	5	-	-	-	-
	Staphylinus sp.	5	3	60	M/O, M, M	4/1, 6, 2
Shimla	*Sciara sp.*	5	2	40	N/M, N/M	2/10, 3/3
Sirmaur	*Sciara sp.*	5	-	-	-	-
	S. nigrofasciatum	5	1	20	N/M	4/4
	Staphylinus sp.	5	2	40	M, M	6, 3
	C. indicus	5	3	60	M/O, M, O	15/2, 26, 2
Solan	*Sciara sp.*	5	2	40	N, N	5, 1
	Staphylinus sp.	5	-	-	-	-
	C. indicus	5	2	40	N/M	4/36
	S. pallidipes	5	2	40	N, N/M	3, 2/2

N= Nematodes M = Mites O= Others

The flies (*Sciara* sp.) and beetles (*S. nigrofasciatum, Staphylinus* sp. and *C indicus* and *S. pallidipes*) were found to serve as carriers of nematodes, mites. Some unidentified organisms were also observed in the washings of these insects especially beetles. Out of five sciarids collected from Bilaspur, only one was found to carry mites on its body as three mites and two unidentified organisms were recovered in its washings. While sciarids collected from Kangra did not carry nematodes/mites, various species of beetles collected from this locality showed 40.0-80.0 per cent phoresy by nematodes and mites. Similar observations were recorded from other locations as well where higher level of phoresy by nematodes/mites/others was observed in coleopterans as compared to dipterans. One specimen of *C. indicus* collected from Solan showed highest number of mites attached to its elytra and legs (36 mites) followed by *Staphylinus* sp. individual collected from Sirmaur having 26 mites clinging to its body. Phoresy by nematodes was comparatively low. The reason could be either the low level of nematode infestation or lower level of phoretic behavior in nematodes as compared to mites.

Among various insects tested for phoresy of nematodes and mites dipteran fly *Sciara* sp. showed minimum phoresy of 17.1 per cent with phoretic intensity of 1.75, 2.75 and 0.5 in mites, nematodes and others, respectively. Phoresy in coleopterans was much higher than dipterans and ranged from 36.0 to 60.0 per cent. Among beetles, highest per cent phoresy was recorded in *C. indicus* in which 60 per cent beetles had the nematodes and mites attached to their bodies. This was followed by *S. nigrofasciatum* and *S. pallidipes* with 40.0 per cent individuals of both carrying the phoretic organisms. Phoresy was least in *Staphylinus* sp. as only 36.0 per cent individuals of this beetle acted as carriers of nematodes and mites. Phoretic intensity was also highest in *C. indicus* in which 18 mites and 1.2 nematodes clinged per beetle followed by *Staphylinus* sp. in which each carrier beetle carried on an average 4.6 mites and 0.8 nematodes (Table 7b).

Table 7 b Frequency and intensity of phoresy in insects associated with mushroom

Insects	No. washed	No. of insect carriers	Per cent phoresy	Clinging of N/M/O	Average Phoretic intensity
***Sciara* sp.**	35	6	17.1	M/N/O	2.7/1.8/0.32
S. nigrofasciatum	10	4	40.0	M/N/O	2.0/5.0/0
***Staphylinus* sp.**	25	9	36.0	M/N/O	4.6/0.8/0.2
C. indicus	15	9	60.0	M/N/O	18/1.2/0.6
S. pallidipes	5	2	40.0	M/N/O	1.0/2.5

N= Nematodes M = Mites O= Others

4.4 Seasonal abundance of sciarid flies in mushroom farms

Perusal of data presented in Table 8 show a wide fluctuation in the populations of flies depending upon the prevailing temperature and Relative humidity during the years 2007-2009. Minimum *Sciara* population ranging from 0-5 was recorded in the month of August during all the three years under observation when average temperature varied from 24.5 to 27.9 ^{o}C and relative humidity from 70.33 to 90.11. As the average temperature and humidity decreased, the population gradually started building up but averagely remained below 250 till average temperature remained below 18 ^{o}C. Peak populations of *Sciara* were observed during months of March, April and May when average temperature ranged from 20.3 to 23.9 ^{o}C and average relative humidity ranged from 54.5 to 64.8 per cent during the years 2008 and 2009. As the temperature started rising beyond

Table 8 Seasonal abundance of *Sciara* sp. in mushroom farms during 2007-2009.

Month	Year 2007					Year 2008					Year 2009				
	Min. temp. (^{O}C)	Max. temp. (^{O}C)	Aver. temp. (^{O}C)	R.H. (%)	Sciarids	Min. temp. (^{O}C)	Max. temp. (^{O}C)	Aver. temp. (^{O}C)	R.H. (%)	Sciarids	Min. temp. (^{O}C)	Max. temp. (^{O}C)	Aver. temp. (^{O}C)	R.H. (%)	Sciarids
Jan						13.6	20.2	16.9	65.22	115	12.3	18.0	15.2	61.36	150
Feb						14.1	21.5	17.8	60.58	225	14.0	20.8	17.4	57.92	225
Mar						21.2	26.3	23.8	56.2	975	18.0	24.7	21.4	54.52	1080
Apr						20.7	25.1	22.9	64.82	820	22.2	25.5	23.9	62.26	810
May						18.1	22.4	20.3	54.47	575	21.0	25.9	23.5	58.36	760
Jun						22.4	29.5	25.9	71.95	105	25.4	28.4	26.9	52.24	82
Jul						23.8	28.5	26.2	77.76	20	24.2	28.1	26.2	69.23	12
Aug	22.8	26.2	24.5	90.11	5	24.9	29.9	27.4	86.22	0	26.1	29.8	27.9	70.33	0
Sep	20.4	25.2	22.8	72.81	18	21.2	26.3	23.8	77.04	32	22.4	27.6	25.0	83.33	15
Oct	18.5	22.8	20.7	65.45	95	18.9	24.8	21.9	69.25	89	18.3	26.2	22.3	62.53	104
Nov	12.8	19.4	16.1	68.25	112	14.2	21.8	18.0	62.64	138	13.6	23.5	18.6	64.2	127
Dec	12.4	18.6	15.5	59.5	110	13.4	20.2	16.8	60.11	175	12.6	21.1	16.9	58.54	75
Corre. (r)	-0.065	0.002	-0.027	-0.542											

24.0 °C, a steep decline in their count was recorded **(Plate 11)**. Virtually no insect was seen in the farm in August month of years 2008 and 2009 when average temperature reached above 27.0 °C. Thus, the temperature range from 20.0-24.0 °C and relative humidity of 54.5 to 64.8 per cent was the best for multiplication of *Sciara* sp.

4.5 Relative susceptibility of mushrooms to *Sciara* sp.

In vitro

4.5.1 Effect on mycelial depletion of *A. bisporus* (Plate 12 a, b, c)

Relative susceptibility of *A. bisporus* against sciarid maggots was evaluated separately. Data contained in Table 9 regarding per cent mycelial depletion by different inoculum levels of sciarid maggots on second, fourth, sixth, eighth and tenth day of inoculation unveiled that per cent mycelial depletion increased with increase in inoculum level as well as duration of time. The minimum mycelial depletion of 5.2 per cent was at inoculation level of two maggots at two days increased to 17.8 per cent at 10 days. The highest inoculum level of 16 maggots caused 29.1 per cent mycelial depletion at two days which enhanced to 63.1 per cent at 10 days. Significant difference in mycelial

Table 9 Effect of different population levels of sciarid maggots on the mycelium of *Agaricus bisporus*

Maggots inoculated	**Per cent mycelial depletion (days)**					**Mean**
	2	**4**	**6**	**8**	**10**	
2	5.2 (13.1)	8.2 (16.7)	13.0 (21.1)	17.5 (24.7)	17.8 (24.9)	**12.3 (20.1)**
4	15.4 (23.1)	19.9 (26.5)	24.6 (29.7)	29.0 (32.5)	34.9 (36.2)	**24.7 (29.6)**
8	21.5 (27.6)	33.1 (35.1)	38.7 (38.5)	42.7 (40.8)	46.1 (42.8)	**36.4 (36.9)**
16	29.1 (32.7)	39.4 (38.8)	50.8 (45.4)	57.0 (49.0)	63.1 (52.7)	**47.9 (43.7)**
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**0.0 (0.0)**
Mean	**14.2 (19.3)**	**20.1 (23.4)**	**25.4 (27.0)**	**29.2 (29.4)**	**32.4 (31.3)**	

Figures in parentheses are arc sine transformed values

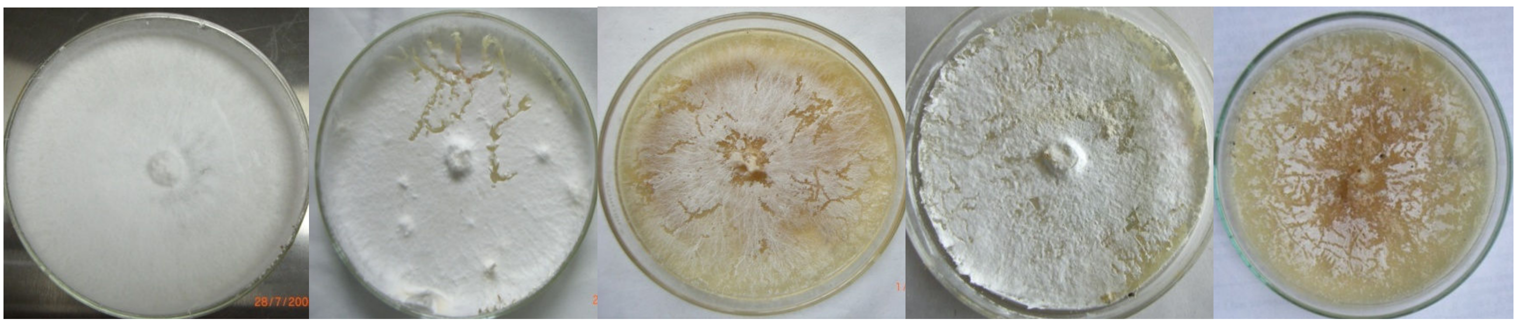
$CD_{(0.05)}$	Maggots (M)	0.61
	Days (D)	0.61
	M x D	1.37

Plate 10 Copulation under captivity conditions

Plate 11 Sciarid flies trapped in yellow sticky traps

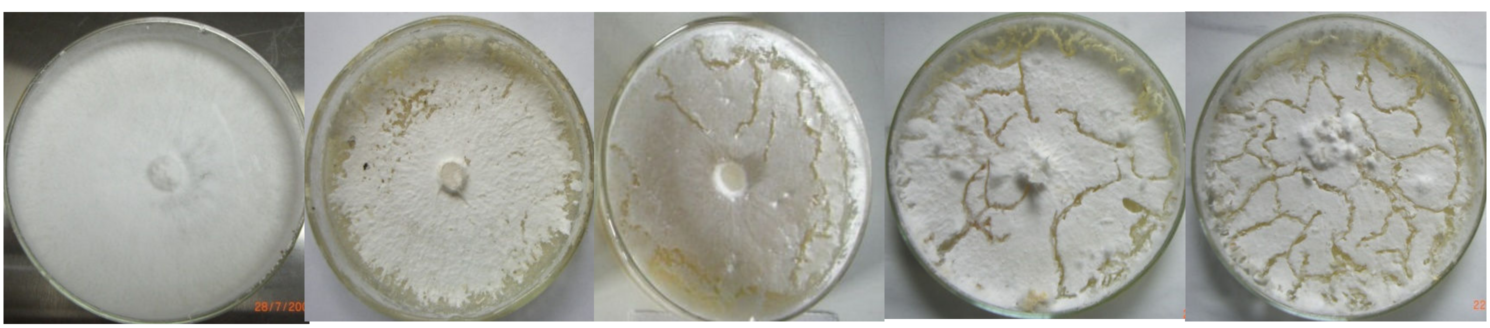
Control 2 larvae 4 larvae 8 larvae 16 larvae

Plate 12.a. Mycelia depletion of *Agaricus bisporus* by sciarid maggots of two days of treatment



Control 2 larvae 4 larvae 8 larvae 16 larvae

Plate 12.b. Mycelia depletion of *Agaricus bisporus* by sciarid maggots of six days of treatment



Control 2 larvae 4 larvae 8 larvae 16 larvae

Plate 12.c. Mycelia depletion of *Agaricus bisporus* by sciarid maggots of 10 days of treatment

depletion was recorded at all the levels of inoculum as well as time intervals. The mean mycelial depletions of 12.3, 24.7, 36.4 and 47.9 per cent caused by respective counts of 2, 4, 8 and 16 maggots were significantly different from each other. Similarly, mean per cent mycelial depletion increased significantly from 14.2 per cent at two days to 32.4 per cent at ten days.

4.5.2 Effect on mycelial depletion of *P. sajor caju* (Plate 13 a, b, c)

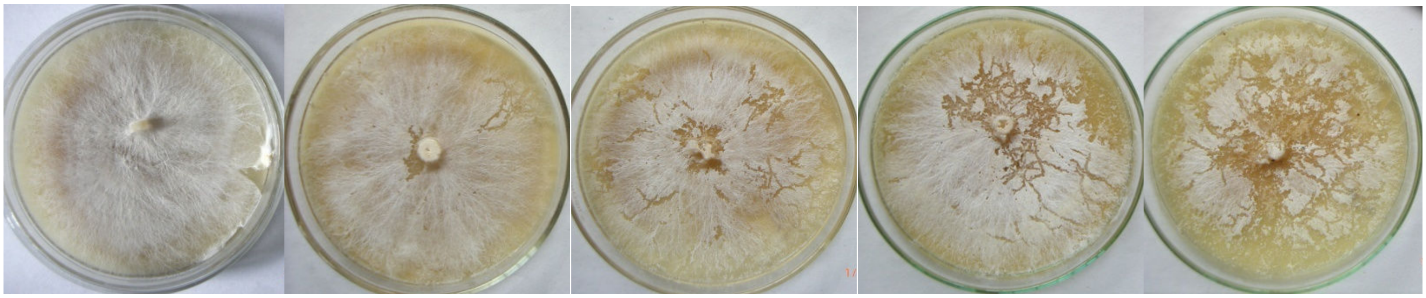
The data presented in Table 10 indicate that mycelial damage increased significantly with increase in larval inoculum as well as duration of time. Mean per cent mycelial depletion increased from 14.9 to 26.1, 40.3 and 48.7 per cent at respective inocula of 2, 4, 8 and 16 maggots. Significantly different mean mycelial depletion of 15.5, 21.9, 26.6, 31.4 and 34.6 per cent were recorded at 2, 4, 6, 8 and 10 days of inoculation, respectively. The minimum mycelial depletion of 5.7 per cent was observed at inoculum level of two maggots at two days which increased to 22.7 per cent at ten days. Maximum injury was caused when 16 maggots were inoculated and mycelial depletion increased from 31.1 per cent at two days to maxima of 64.4 per cent at ten days.

Table 10 Effect of different population levels of sciarid maggots on the mycelium of *Pleurotus sajor caju*

Maggots inoculated	**Per cent mycelial depletion (days)**					**Mean**
	2	**4**	**6**	**8**	**10**	
2	5.7 (13.8)	10.0 (18.4)	15.4 (23.1)	20.7 (27.1)	22.7 (28.5)	**14.9 (22.2)**
4	16.6 (24.0)	21.6 (27.7)	25.8 (30.5)	31.8 (34.3)	34.6 (36.0)	**26.1 (30.5)**
8	24.0 (29.3)	35.7 (36.7)	42.0 (40.4)	48.4 (44.1)	51.2 (45.7)	**40.3 (39.2)**
16	31.1 (33.9)	42.5 (40.7)	49.7 (44.8)	55.9 (48.4)	64.4 (53.3)	**48.7 (44.2)**
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**0.0 (0.0)**
Mean	**15.5 (20.2)**	**21.9 (24.7)**	**26.6 (27.8)**	**31.4 (30.8)**	**34.6 (32.7)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Maggots (M) 0.49
Days (D) 0.49
M x D 1.10



Control 2 larvae 4 larvae 8 larvae 16 larva

Plate 13.a. Mycelia depletion of *Pleurotus sajor caju* by *Sciara* maggots of two days of treatment

Control 2 larvae 4 larvae 8 larvae 16 larvae

Plate 13.b. Mycelia depletion of *Pleurotus sajor caju* by *Sciara* maggots of six days of treatment

Control 2 larvae 4 larvae 8 larvae 16 larvae

Plate 13.c. Mycelia depletion of *Pleurotus sajor caju* by *Sciara* maggots of 10 days of treatment

4.6 Damaging potential of *Sciara* sp. against *A. bisporus*

In vivo

4.6.1 Effect on mycelial depletion

Observations regarding effect of different population levels of maggots of *Sciara* sp. on the mycelial depletion of white button mushrooms, when inoculated at different stages of cropping have been depicted in Tables 11-14. Data presented in Table 11 signifies the effect of inoculum level as well as inoculation time on the mycelial depletion of *A. bisporus*. Observation recorded at casing time showed minimum mean mycelial damage of 1.7 per cent at inoculum level of two maggots which was at par with 2.1 per cent in uninoculated control. The mycelial depletion increased with enhancement of larval inoculum irrespective of the inoculation time. Mean per cent mycelial depletion of 2.8 per cent at inoculum level of two maggots increased to the tune of 14.9 per cent at the inoculum level of 200 maggots. There was non significant difference in the mean mycelial depletion when maggots were inoculated at spawning or spawning + casing with respective mean mycelial depletion of 9.7 and 10.3 per cent. Mycelial depletion in casing time inoculations was insignificant and at par with un-inoculated control.

Table 11 Effect of different population levels of *Sciara* maggots on the mycelium of *Agaricus bisporus* at casing time

Inoculation time	Mycelial depletion (%)				Mean
	2 maggots	20 maggots	200 maggots	Un-inoculated control	
Spawning	3.7 (10.9)	12.0 (20.2)	21.2 (27.4)	2.1 (8.2)	**9.7 (16.9)**
Casing	1.7 (7.5)	2.2 (8.5)	2.5 (9.2)	2.1 (8.2)	**2.1 (8.4)**
Spawning +Casing	3.1 (10.2)	14.9 (22.6)	21.1 (27.4)	2.1 (8.2)	**10.3 (17.1)**
Mean	**2.8 (9.6)**	**9.7 (17.1)**	**14.9 (21.3)**	**2.1 (8.2)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$	Inoculation time (I)	1.3
	Maggots (M)	1.5
	I x M	2.6

Observations regarding mycelial depletion after 15 days of casing as depicted in Table 12 revealed the fact that damage to the mycelium increased with time and reached to the tune of 78.3 per cent when 200 *Sciara* maggots were inoculated each at spawning and casing. The mycelium used for sporophore development in an uninoculated control at this time was 38.4 per cent. A positive trend of mycelial depletion was recorded with increase in the inocula. The mean mycelial inhibition of 46.6, 57.2 and 71.7 per cent were recorded at respective inoculum levels of 2, 20 and 200 maggots. The maggots inoculated at spawning or casing time caused significantly lower mean mycelial depletion (49.8 and 53.1 per cent, respectively) than those inoculated at spawning as well as casing causing mean mycelial inhibition of 57.5 per cent at this time. However, interaction between inoculum level and inoculation time was found to be non significant.

Table 12 Effect of different population levels of *Sciara* maggots on the mycelium of *Agaricus bisporus* at 15 days of casing

Inoculation time	**Mycelial depletion (%)**				**Mean**
	2 maggots	**20 maggots**	**200 maggots**	**Un-inoculated control**	
Spawning	46.7 (43.1)	56.9 (48.9)	70.5 (57.1)	38.4 (38.3)	**53.1 (46.9)**
Casing	43.9 (41.5)	50.5 (45.3)	66.2 (54.4)	38.4 (38.3)	**49.8 (44.9)**
Spawning +Casing	49.1 (44.5)	64.2 (53.3)	78.3 (62.3)	38.4 (38.3)	**57.5 (49.6)**
Mean	**46.6 (43.0)**	**57.2 (49.2)**	**71.7 (57.9)**	**38.4 (38.3)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$	Inoculation time (I)	2.3
	Maggots (M)	2.6
	I x M	N.S.

Data recorded in Table 13 at 30 days of casing showed 58.0 per cent utilization of the mycelium for sporophore development in uninoculated bags. As compared to this, the maximum depletion of 94.1 per cent was observed in bags receiving 200 maggots each at spawning as well as casing time. At the lowest inoculum level of two maggots, per cent mycelial depletion of 68.7 to 72.4 per cent was statistically alike irrespective of inoculation time. At inoculum level of 20 maggots, the mycelial depletion in spawning and spawning + casing time was statistically similar (79.9 and 87.5 per cent) and was significant more than casing time inoculation (72.2 per cent). Interestingly at the highest inoculum level of 200 maggots, spawning and casing time inoculation caused significantly similar mycelial depletion of 84.5 and 87.6 per cent, respectively. These values were significantly lower than 94.1 per cent inhibition caused when maggots were inoculated at spawning as well as casing. Similarly, mean mycelial depletions of 73.5 and 70.6 per cent at respective spawning and casing time inoculations were statistically at par and were significantly lower than 78.0 per cent when maggots were inoculated at spawning as well as casing time. However, levels of inocula showed a significant difference as mean mycelial depletion of 69.5, 79.9 and 88.9 per cent were recorded at respective levels of 2, 20 and 200 maggots.

Table 13 Effect of different population levels of *Sciara* maggots on the mycelium of *Agaricus bisporus* at 30 days of casing

Inoculation time	Mycelial depletion (%)				Mean
	2 maggots	20 maggots	200 maggots	Uninoculated control	
Spawning	68.7 (55.9)	79.9 (63.4)	87.6 (69.4)	58.0 (49.6)	**73.5 (59.6)**
Casing	67.5 (55.3)	72.2 (58.2)	84.5 (66.8)	58.0 (49.6)	**70.6 (57.5)**
Spawning +Casing	72.4 (58.3)	87.5 (63.6)	94.1 (76.8)	58.0 (49.6)	**78.0 (63.5)**
Mean	**69.5 (56.5)**	**79.9 (63.6)**	**88.9 (71.0)**	**58.0 (49.6)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$	Inoculation time (I)	2.3
	Maggots (M)	2.6
	I x M	4.6

Observations as recorded on mycelial depletion at 45 days of casing i.e. at the termination of the experiment have been presented in Table 14. Data exposed the minimum and maximum inhibition of 89.9 and 99.5 per cent at respective inoculum levels of two casing time larval inoculations and 200 spawning + casing time inoculations, respectively. Mycelial utilization in uninoculated control at this time was 80.3 per cent that occurred due to development of sporophores from the mycelium. There was no significant difference in the mean mycelial depletion when inoculations were made at spawning or casing (90.5 and 90.7 per cent, depletion. respectively). However, the bags inoculated at spawning as well as casing showed significantly higher mean mycelial depletion of 93.1 per cent which was significantly higher than the former as well as uninoculated control. The level of inocula played a significant role as statistically different mean mycelial damage of 91.5, 95.9 and 98.0 per cent was observed when 2, 20 and 200 maggots were inoculated, respectively.

Table 14 Effect of different population levels of *Sciara* maggots on the mycelium of *Agaricus bisporus* at 45 days of casing (termination of the experiment)

Inoculation time	Mycelial depletion (%)				Mean
	2 maggots	20 maggots	200 maggots	Uninoculated control	
Spawning	90.7 (72.3)	94.0 (75.9)	97.1 (80.3)	80.3 (63.6)	**90.5 (73.0)**
Casing	89.9 (71.6)	94.9 (77.6)	97.6 (81.3)	80.3 (63.6)	**90.7 (73.5)**
Spawning + Casing	93.8 (75.9)	98.8 (79.1)	99.5 (82.7)	80.3 (63.6)	**93.1 (77.5)**
Mean	**91.5 (73.3)**	**95.9 (79.1)**	**98.0 (82.7)**	**80.3 (63.6)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$	Inoculation time (I)	2.2
	Maggots (M)	2.6
	I x M	N.S.

4.6.2 Multiplication potential of *Sciara* sp. on *A. bisporus*

The data multiplication of *Sciara* sp. in relation to their initial population as recorded at casing and 15, 30 and 45 days of casing (termination of experiment) have been presented vide Tables 15-17.

As evident from Table 15 that included information on multiplication of *Sciara* sp. when inoculated at spawning time, the insect population increased proportionately to their initial inocula. Final mean population of 1.2, 2.3 and 5.1 recorded at respective initial inocula of 2, 20 and 200 were significantly different from each other. The number increased with increase in the time interval as mean larval population of 1.4 at casing increased significantly to 4.4, 6.7 and 10.0 at 15, 30 and 45 days of casing, respectively. Almost similar trends were observed with other stages of development like pre-pupae and pupae. Adult population was not counted due to their flying behavior.

As clear from observations recorded in Table 16, sciarids when inoculated at casing time showed a progressive multiplication in the cropping bags till the experiment was terminated. The mean larval population of 3.8 at 15 days of casing touched the level of 8.6 by the time the cropping ceased. Population of other stages like pre-pupae and pupae also increased correspondingly. Since,

Table 15 Multiplication potential of *Sciara* sp. on *A. bisporus* (spawning time inoculation)

Larval inoculum	Population at (per 250 cc sample)												MEAN
	Casing time			15 days of Casing			30 days of Casing			45 days of Casing			
	M	PP	P	M	PP	P	M	PP	P	M	PP	P	
2	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	1.3 (1.5)	0.7 (1.3)	0.3 (1.1)	2.3 (1.8)	0.7 (1.3)	1.3 (1.5)	4.3 (2.3)	1.3 (1.5)	2.3 (1.8)	**1.2 (1.4)**
20	1.0 (1.4)	0.0 (1.0)	0.0 (1.0)	3.3 (2.1)	1.3 (1.5)	1.7 (1.6)	4.7 (2.4)	1.0 (1.4)	1.7 (1.6)	7.3 (2.9)	2.3 (1.8)	3.7 (2.2)	**2.3 (1.7)**
200	3.3 (2.1)	0.0 (1.0)	0.3 (1.1)	8.7 (3.1)	2.0 (1.7)	3.3 (2.1)	13.0 (3.7)	1.7 (1.6)	2.0 (1.7)	18.3 (4.4)	3.7 (2.2)	4.3 (2.3)	**5.1 (2.3)**
MEAN	**1.4 (1.5)**	**0.0 (1.0)**	**0.1 (1.1)**	**4.4 (2.2)**	**1.3 (1.5)**	**1.8 (1.6)**	**6.7 (2.7)**	**1.1 (1.4)**	**1.7 (1.6)**	**10.0 (3.2)**	**2.4 (1.8)**	**3.4 (1.8)**	

M: Maggot **PP: Pre-pupa** **P: Pupa**

Figures in parentheses are square root (√x+1) transformation values

$CD_{0.05}$:

Maggots inoculated (M)	0.10
Intervals(I)	0.11
Population(P)	0.12
M x I x P	N.S.

Table 16 Multiplication potential of *Sciara* sp. on *A. bisporus* (casing time inoculation)

Larval inoculum	Population at (per 250 cc sample)									MEAN
	15 days of Casing			30 days of Casing			45 days of Casing			
	M	**PP**	**P**	**M**	**PP**	**P**	**M**	**PP**	**P**	
2	1.0 (1.4)	0.3 (1.1)	0.3 (1.1)	1.7 (1.6)	0.7 (1.3)	0.3 (1.1)	3.7 (2.2)	1.0 (1.4)	1.3 (1.4)	1.2 (1.4)
20	2.7 (1.9)	1.0 (1.4)	1.3 (1.5)	3.7 (2.2)	0.7 (1.3)	1.0 (1.4)	6.3 (2.7)	1.7 (1.6)	2.3 (1.8)	2.3 (1.8)
200	7.7 (2.9)	1.3 (1.5)	2.7 (1.9)	11.0 (3.5)	1.3 (1.5)	1.7 (1.6)	15.7 (4.1)	2.7 (1.9)	4.0 (2.2)	5.3 (2.4)
MEAN	3.8 (2.1)	0.9 (1.4)	1.4 (1.5)	5.5 (2.4)	0.9 (1.4)	1.0 (1.4)	8.6 (2.9)	1.8 (1.7)	2.6 (1.9)	

M: Maggot **PP: Pre-pupa** **P: Pupa**

Figures in parentheses are square root ($\sqrt{x+1}$) transformation values

$CD_{0.05}$:	Maggots inoculated (M)	0.18
	Intervals (I)	0.11
	Population (P)	0.16
	M x I x P	0.30

Table 17 Multiplication potential of *Sciara* sp. on *A. bisporus* (spawning + casing time inoculation)

Larval inoculum	Population at (per 250 cc sample)												MEAN
	Casing time			15 days of Casing			30 days of Casing			45 days of Casing			
	M	PP	P	M	PP	P	M	PP	P	M	PP	P	
2	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	2.3 (1.8)	1.3 (1.5)	2.0 (1.7)	3.7 (2.2)	1.7 (1.6)	2.0 (1.7)	6.0 (2.6)	2.0 (1.7)	3.3 (2.1)	**2.0 (1.7)**
20	1.3 (1.5)	0.0 (1.0)	0.0 (1.0)	4.0 (2.2)	1.7 (1.6)	2.7 (1.9)	7.3 (2.9)	2.0 (1.7)	2.3 (1.8)	12.3 (3.6)	2.3 (1.8)	4.7 (2.4)	**3.4 (1.9)**
200	4.3 (2.3)	0.0 (1.0)	0.7 (1.4)	13.0 (3.7)	2.3 (1.8)	5.3 (2.5)	16.3 (4.2)	2.3 (1.8)	3.0 (1.9)	25.3 (5.1)	3.7 (2.1)	5.3 (2.5)	**6.8 (2.5)**
MEAN	**1.9 (1.6)**	**0.0 (1.0)**	**0.2 (1.1)**	**6.4 (2.6)**	**1.8 (1.7)**	**3.3 (2.0)**	**9.1 (3.1)**	**2.0 (1.7)**	**2.4 (1.8)**	**14.6 (3.8)**	**3.9 (1.9)**	**4.5 (2.3)**	

M: Maggot **PP: Pre-pupa** **P: Pupa**

Figures in parentheses are square root ($\sqrt{x+1}$) transformation values

$CD_{0.05}$:	Maggots inoculated (M)	0.10
	Intervals (I)	0.13
	Population(P)	0.11
	M x I x P	N.S.

the insect completed its life cycle in about 20 days, a big number of adults also emerged during the cropping phase but their counts could not be taken as they moved from one bag to the other. Multiplication of the insect was density dependent and highest multiplication rate at this cessation of crop was observed at the lowest initial inoculum.

Interpretations made from the data presented in Table 17 were indicative of high multiplication of insect maggots in *A. bisporus* when different populations of the test insect were inoculated during spawning as well as casing phase of cropping. The initial count of two maggots inoculated each at spawning and casing/bag reached to the tune of 6.0/ 250 cc by the end of cropping. Other stages were also found in varying numbers in compost/ casing samples analyzed from time to time. Corresponding higher populations were recorded at the higher inocula.

4.6.3 Effect of different population levels of *Sciara* maggots on sporophore yields of *Agaricus bisporus*

Statistically scrutinized data on effect of *Sciara* maggots on sporophore yield of *A. bisporus* have been presented through Tables 18-20.

4.6.3.1 Effect on total sporophore yield (Plate 14-16)

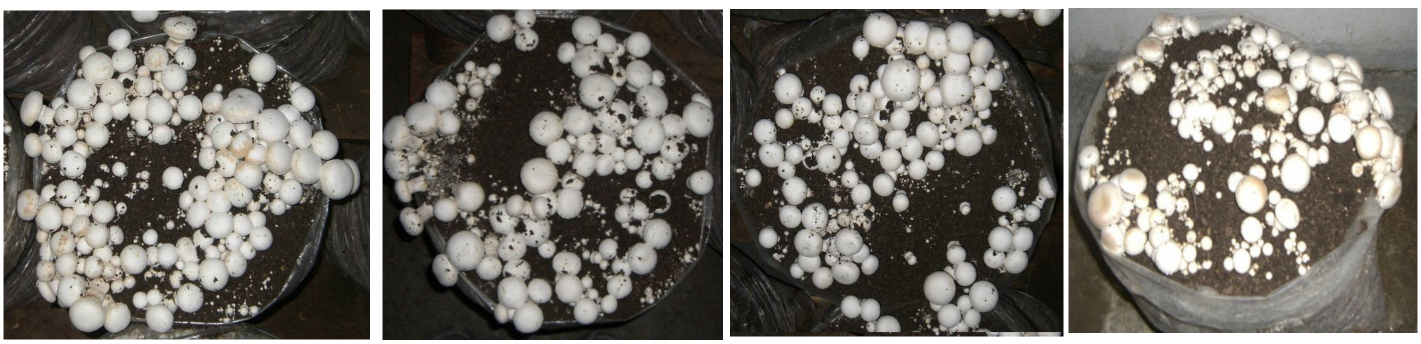
Data presented in Table 18 depict the efficiency of *Sciara* maggots as mushroom pests when inoculated at spawning time. Highest sporophore yield of 1706.7 g/bag in uninoculated control was significantly higher than that recorded in the insect inoculated bags. A progressive decline in button production from 1480.0, 1168.3 to 800.0 g/bag was recorded at respective inocula of 2, 20 and 200 maggots. Data clearly indicated that inoculum level and time of larval inoculation had a great impact on sporophore production of white button mushrooms. Losses to the tune of 13.3, 7.1 and 18.7 per cent

Table 18 Effect of different inoculation levels of *Sciara* maggots on sporophore yield of *A. bisporus* (spawning time inoculation)

Maggots inoculated	Mean Sporophore yield (g)						Total yield (g)	Loss (%)
	1st week	2nd week	3rd week	4th week	5th week	6th week		
2	300.0	406.7	275.0	235.0	151.7	111.7	1480.0	13.3
20	221.7	330.0	206.7	181.7	131.7	96.7	1168.3	31.5
200	197.7	142.3	147.3	127.7	105.0	80.0	800.0	53.1
Control	330.0	473.3	298.3	281.7	170.0	153.3	1706.7	-
$CD_{(0.05)}$	**N.S.**	**63.4**	**40.3**	**79.1**	**40.9**	**56.1**	**99.7**	

Control **2 larvae** **20 larvae** **200 larvae**

Plate 14 Effect of sciarid larvae in *Agaricus bisporus* (spawning time inoculation)



Control **2 larvae** **20 larvae** **200 larvae**

Plate 15 Effect of sciarid larvae in *Agaricus bisporus* (casing time inoculation)

Control **2 larvae** **20 larvae** **200 larvae**

Plate 16 Effect of sciarid larvae in *Agaricus bisporus* (spawning + casing time inoculation)

over control were incurred when two maggots were inoculated at spawning, casing and spawning + casing time, respectively. The corresponding decline in yields with inoculum level of 20 maggots were 31.5 (Table 18), 24.2 (Table 19) and 42.8 (Table 20) per cent. Highest yield losses of 56.7 per cent were suffered at treatment level of 200 maggots inoculated at spawning + casing (Table 20) followed by 53.1 (Table 18) and 49.9 (Table 19) per cent losses incurred by this inoculum when applied at spawning and casing, respectively.

Table 19 Effect of different inoculation levels of *Sciara* maggots on sporophore yield of *A. bisporus* (casing time inoculation)

Maggots inoculated	Mean Sporophore yield (g)						Total yield (g)	Loss (%)
	1st week	2nd week	3rd week	4th week	5th week	6th week		
2	370.0	473.3	300.0	188.3	136.7	118.3	1586.7	7.1
20	276.7	401.7	220.0	158.3	128.3	110.0	1295.0	24.2
200	256.7	190.0	151.0	100.0	83.3	75.0	856.0	49.9
Control	330.0	473.3	298.3	281.7	170.0	153.3	1706.7	-
$CD_{(0.05)}$	**N.S.**	**65.9**	**41.6**	**47.0**	**52.1**	**53.0**	**96.2**	

Table 20 Effect of different inoculation levels of *Sciara* maggots on sporophore yield of *A. bisporus* (spawning + casing time inoculation)

Maggots inoculated	Mean Sporophore yield (g)						Total yield (g)	Loss (%)
	1st week	2nd week	3rd week	4th week	5th week	6th week		
2	305.0	430.0	265.0	218.3	101.7	68.3	1388.3	18.7
20	201.7	381.7	150.0	106.7	76.3	60.0	976.3	42.8
200	215.0	178.3	153.3	105.0	50.0	38.3	740.0	56.7
Control	330.0	473.3	298.3	281.7	170.0	153.3	1706.7	-
$CD_{(0.05)}$	**N.S.**	**77.3**	**56.5**	**62.9**	**46.1**	**25.5**	**113.0**	

4.6.3.2 Effect on flush pattern

Since, buttons of *A. bisporus* normally appear in succession of flushes at a gap of seven to eight days, the weekly sporophore production per treatment was assessed till sixth week when further maintenance of bags in most of the treatments became economically unviable. Weekly yield as interpreted in different treatments have been depicted in Figures 8-10. Normal flush pattern of alternate low and high yields during initial stages of cropping for first two weeks followed by steady decline in

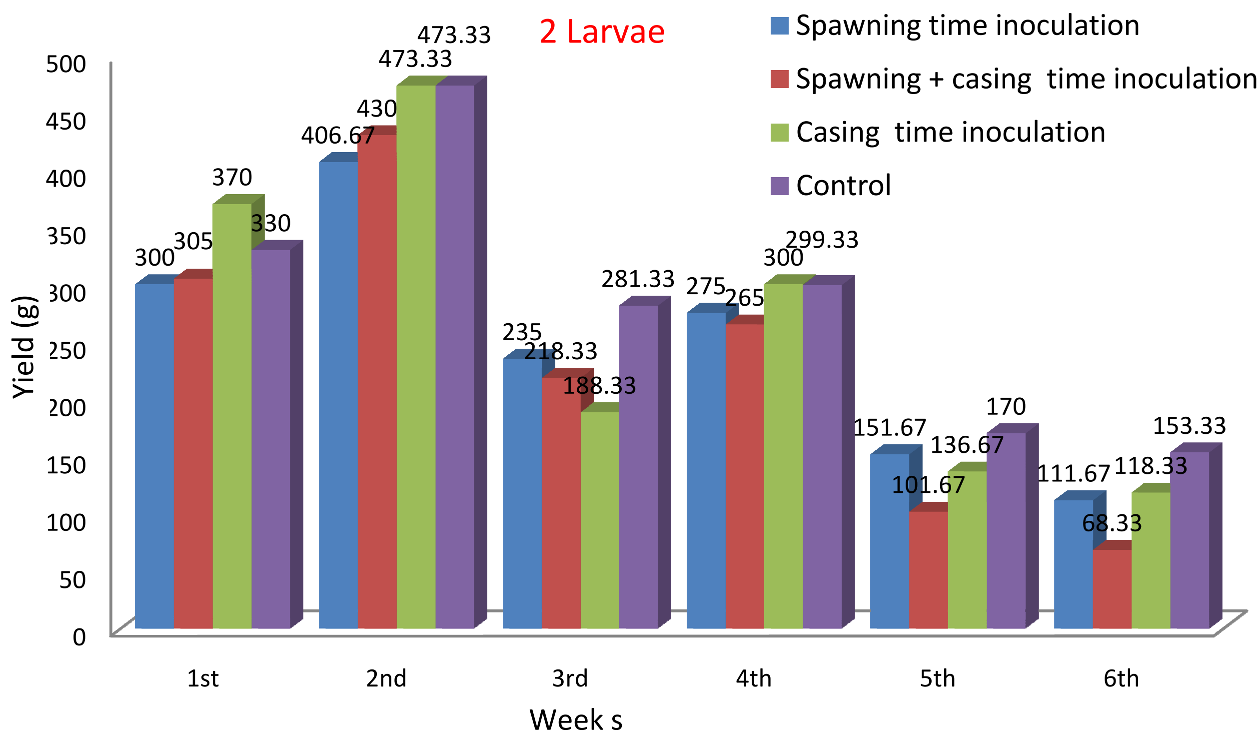


Fig 8 Effect of different time inoculations of two sciarid larvae on flush pattern of *A. bisporus*

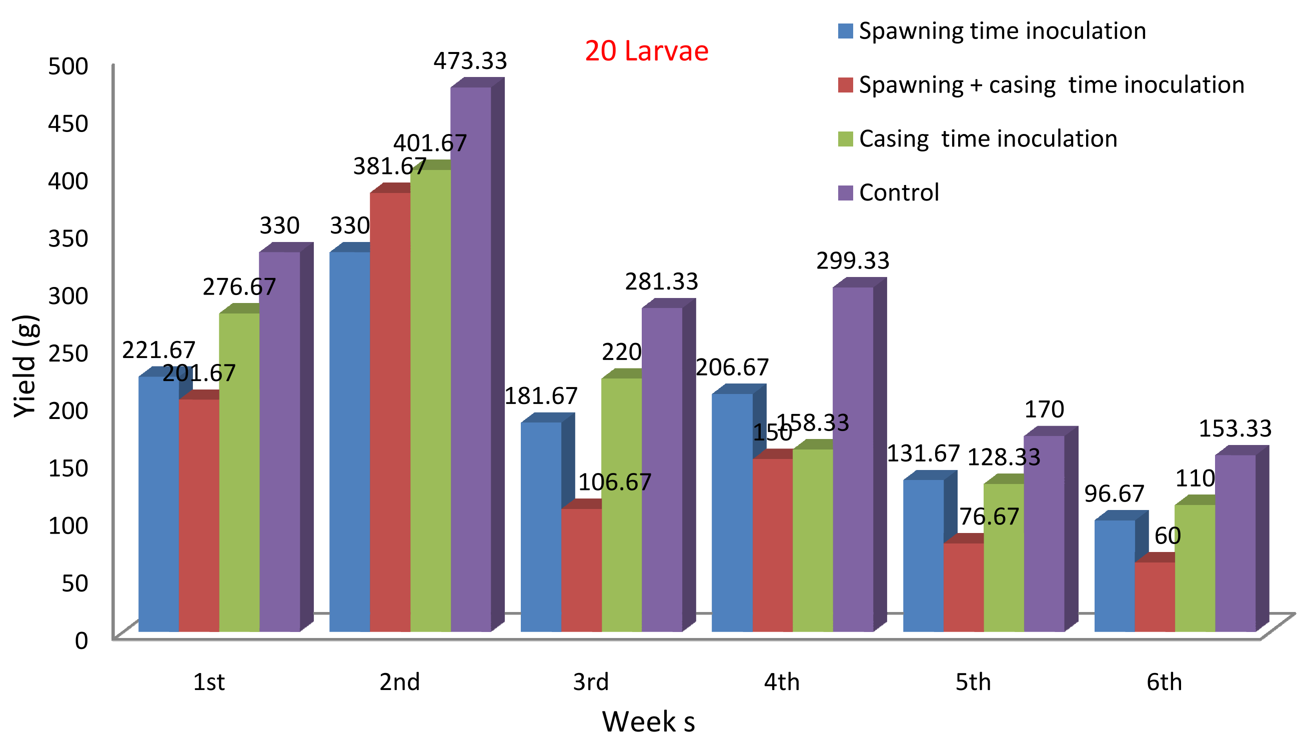


Fig 9 Effect of different time inoculations of 20 sciarid larvae on flush pattern of *A. bisporus*

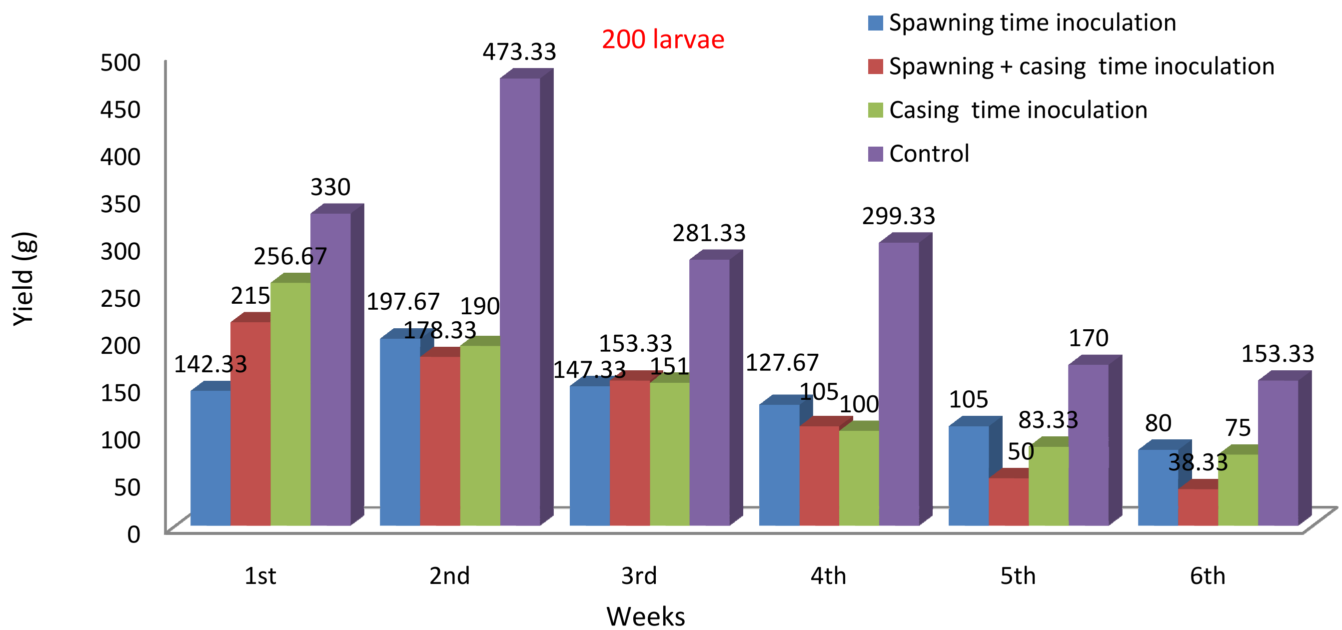


Fig 10 Effect of different time inoculations of 200 sciarid larvae on flush pattern of *A. bisporus*

button production was recorded in uninoculated control as well as lowest inocula of two maggots, irrespective of the inoculation stage (Fig. 8). The pattern of button emergence was nearly same at the inoculum level of 20 maggots but for the significantly poor yields in all the treatments as compared to uninoculated check at all stages of cropping (Fig. 9). The flush pattern was disturbed at the highest inoculum of 200 maggots. The sporophore emergence was extremely low as compared to control at all the inoculation times. Contrary to low and high yields in succession of flushes in the early stages of cropping in uninoculated bags, a continuous decline in sporophore emergence was recorded with the progress in time in all the treatments up to fourth week. Maintenance of bags then onwards became economically unviable as sporophore yield obtained per bag was 100g or lower beyond this period (**Fig. 10**).

4.6.3.3 Correlation between larval population, mycelial population and sporophore yield

As evident from Table 21, a highly positive correlation between the larval population and mycelial depletion existed in all the treatments irrespective of the time of inoculation. A negative correlation between insect count and sporophore yields was also evident. Similarly, mycelial depletion and sporophore yields were negatively correlated i.e. higher the mycelial depletion lower was the button production.

Table 21 Correlation between insect population, mycelia depletion and sporophore yield in *A. bisporus*

	Yield	**Mycelial depletion at spawning**	**Mycelial depletion at casing**	**Mycelial depletion at spawning + casing**	**Larval Population**
Yield	1.0000				
Mycelial depletion at spawning	-0.8418*	1.0000			
Mycelial depletion at casing	-0.7262*	0.9898*	1.0000		
Mycelial depletion at spawning + casing	-0.7804*	0.9417*	0.9382*	1.0000	
Larval Population	-0.9421*	0.9483*	0.9128*	0.8662*	1.0000

*Significant at 5 % level of confidence

4.6.3.4 Symptomatology

The visual symptoms of insect damage were observed on pin heads as well as fruiting bodies. The data on per cent healthy/damaged sporophores at different inocula and different stages of inoculation have been shown in Table 22.

Whereas, more than 99 per cent buttons produced in control bags were healthy, highest per cent deterioration was seen at inoculum level of 200 maggots where only 79.6 per cent sporophores were healthy when maggots were inoculated at spawning as well as casing time. Per cent of healthy sporophores at this inoculum level in spawning and casing time inoculation was 85.0 and 87.78 per cent, respectively. This per cent of healthy sporophores was significantly higher than the former. However, at inoculum level of 20 maggots, counts of healthy sporophores in spawning and spawning + casing time inoculation were statistically at par (86.8 and 85.2 per cent, respectively) but were significantly lower as compared to 91.4 per cent healthy sporophores observed in treatment receiving casing time inoculations. Overall, the inoculum levels of 2 and 20 maggots incurred statistically similar visual deterioration with mean value of 88.9 and 87.8 per cent healthy sporophores as compared to significantly lower 84.1 per cent at the inoculum level of 200 maggots. However, all these values were significantly lower than mean per cent value of 99.6 achieved in untreated bags. Cropping stage at which the insect first invaded, played a vital role as significantly different number of healthy sporophores to the tune of mean per cent values of 90.5, 92.4 and 87.4 were observed at respective inoculation times of spawning, casing and spawning + casing.

Table 22 Effect of different inocula of *Sciara* maggots on morphology of *A. bisporus* sporophores

Maggots inoculated	Healthy sporophores (%)			Mean
	Spawning time inoculation	Casing time inoculation	Spawning + casing time inoculation	
2	90.4 (71.9)	91.1 (73.0)	85.0 (67.3)	**88.9 (70.8)**
20	86.8 (68.6)	91.4 (72.9)	85.2 (67.4)	**87.8 (69.6)**
200	85.0 (67.3)	87.7 (69.4)	79.6 (63.2)	**84.1 (66.6)**
Control	99.7 (88.1)	99.3 (86.1)	99.7 (88.1)	**99.6 (87.4)**
Mean	**90.5 (73.9)**	**92.4 (75.4)**	**87.4 (71.5)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Inoculation time(I) 1.5
Maggots (M) 1.7
I x M 2.9

4.6.3.5 Visual symptoms caused by *Sciara* sp. on *Agaricus bisporus* sporophores

Visual symptoms were first evident at pin head formation stage where insect infested pin heads looked brown as compared to pure white healthy ones. These brown pin heads dried out prematurely and never developed into fruiting bodies. Button production was uneven due to patchy mycelial impregnation. The infested sporophores wore an unhealthy look as they were slightly brownish and shriveled with a typical 'Fly speck' on the cap. Visual tunneling was very clear in the dissected heavily infested buttons caused by the movement and feeding of maggots that entered from the base of stripe and moved towards the pileus of the emerging sporophores.

4.7. *P. sajor caju*

4.7.1 Multiplication potential of *Sciara* sp.

It is clear from data cited in Table 23 that all the developmental stages of pest barring adults were found in the substrate medium of the oyster mushroom. The adults despite their presence in abundance could not be counted due to their flying behavior. The pest population increased with increase in inoculum level and with progress of time. The initial population of 2, 20 and 200 per bag reached the respective mean populations of 1.9, 3.4 and 5.1per 250 cc samples. Despite of the fact that insect population multiplied with increase in inoculum level, the corresponding rate of multiplication was reduced with increase in inoculum level. Similarly, the mean population of 1.8 maggots, 0.1 pre pupae and 0.1 pupae at pin head stage attained the number equivalent to 13.0 maggots, 3.3 pre pupae and 4.3 pupae at the time of termination.

Observations regarding Sciarids population when they infested *P. sajor caju* crop at late stage of pin head formation were recorded at 15, 30 and 45 days of pin head formation and have been shown in Table 24. Negligible population was observed at 15 days of pin head formation. As the life cycle completed, the insect count increased and various stages of insect could be seen in the substrate medium at 30 days of pin head formation. A significant increase in all stages of insect population was recorded at 40 days of pin head formation with respect to population at 30 days. Mean larval, pre-pupal and pupal count of 7.3, 1.3 and 2.2 at 30 days at pin head formation time touched the respective maxima of 10.6, 1.8 and 3.6 at 45 days of pin head formation time. The initial level of inoculum affected the final population significantly. The initial inocula of 2, 20 and 200 sciarid maggots produced the

Table 23 Multiplication potential of *Sciara* sp. on *P. sajor caju* (spawning time inoculation)

Larval inoculum	Population at (per 250cc sample)												MEAN
	Pin head formation			15 days of Pin head			30 days of Pin head		45 days of Pin head				
	M	PP	P	M	PP	P	M	PP	P	M	PP	P	
2	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	2.3 (1.8)	0.7 (1.3)	0.7 (1.3)	3.7 (2.2)	1.3 (1.5)	2.3 (1.8)	6.0 (2.6)	2.0 (1.7)	3.7 (2.2)	**1.9 (1.4)**
20	1.3 (1.5)	0.0 (1.0)	0.0 (1.0)	4.0 (2.2)	1.0 (1.4)	2.3 (1.8)	7.3 (2.9)	2.0 (1.7)	3.0 (1.9)	12.3 (3.6)	3.3 (2.1)	4.3 (2.3)	**3.4 (1.9)**
200	4.0 (2.2)	0.3 (1.1)	0.3 (1.1)	10.0 (3.3)	1.3 (1.5)	4.3 (2.3)	15.3 (4.0)	2.3 (1.8)	3.3 (2.1)	20.7 (4.7)	4.7 (2.4)	5.0 (2.4)	**5.1 (2.4)**
MEAN	**1.8 (1.6)**	**0.1 (1.1)**	**0.1 (1.1)**	**5.4 (2.5)**	**1.0 (1.4)**	**2.4 (1.8)**	**8.8 (3.0)**	**1.9 (1.7)**	**2.9 (1.9)**	**13.0 (3.6)**	**3.3 (2.1)**	**4.3 (2.3)**	

M: Maggot **PP: Pre-pupa** **P: Pupa**

Figures in parentheses are square root (√x+1) transformation values

$CD_{0.05}$:

	Maggots inoculated (M)	0.11
	Intervals(I)	0.16
	Population(P)	0.12
	M x I x P	N. S.

Table 24 Multiplication potential of *Sciara* sp. on *P. sajor caju* (Pin head formation time inoculation)

Larval inoculum	Population at (per 250cc sample)									MEAN
	15 days of Pin head			30 days of Pin head		45 days of Pin head				
	M	PP	P	M	PP	P	M	PP	P	
2	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	2.7 (1.9)	0.7 (1.3)	1.7 (1.6)	4.7 (2.4)	1.7 (1.6)	2.7 (1.9)	**1.9 (1.4)**
20	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	6.0 (2.6)	1.3 (1.5)	2.3 (1.8)	8.7 (3.1)	2.3 (1.8)	3.7 (2.1)	**3.3 (1.9)**
200	1.0 (1.4)	0.3 (1.1)	0.3 (1.1)	13.3 (3.8)	2.0 (1.7)	2.7 (1.9)	18.3 (4.4)	3.0 (1.9)	4.3 (2.3)	**6.2 (2.5)**
MEAN	**0.3 (2.2)**	**0.1 (1.3)**	**0.1 (1.7)**	**7.3 (2.8)**	**1.3 (1.5)**	**2.2 (1.8)**	**10.6 (3.3)**	**1.8 (1.8)**	**3.6 (2.1)**	

M: Maggot **PP: Pre-pupa** **P: Pupa**

Figures in parentheses are square root (√x+1) transformation values

$CD_{0.05}$:	Maggots inoculated (M)	0.12
	Intervals (I)	0.12
	Population (P)	0.11
	M x I x P	0.35

Table 25 Multiplication potential of *Sciara* sp. on *P. sajor caju* (spawning + pin head formation time inoculation)

Larval inoculum	Population at (per 250cc sample)												MEAN
	Pin Head Formation			15 days of Pin Head Formation			30 days of Pin Head Formation		45 days of Pin Head Formation				
	M	PP	P	M	PP	P	M	PP	P	L	PP	P	
2	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	3.3 (2.1)	1.0 (1.4)	1.3 (1.5)	5.0 (2.4)	1.7 (1.6)	3.0 (1.9)	7.7 (2.9)	1.7 (1.6)	4.3 (2.3)	**2.4 (1.8)**
20	1.3 (1.5)	0.0 (1.0)	0.0 (1.0)	5.0 (2.4)	1.3 (1.5)	2.7 (1.9)	8.7 (3.1)	2.7 (1.9)	3.3 (2.1)	14.3 (3.9)	3.0 (1.9)	5.0 (2.4)	**3.9 (2.1)**
200	4.7 (2.4)	0.3 (1.1)	0.7 (1.3)	11.3 (3.5)	2.0 (1.7)	4.7 (2.4)	17.0 (4.2)	3.3 (2.1)	4.3 (2.3)	23.7 (4.9)	4.3 (2.3)	5.7 (2.6)	**6.8 (2.6)**
MEAN	**2.0 (1.6)**	**0.1 (1.1)**	**0.2 (1.1)**	**6.6 (2.7)**	**1.4 (1.6)**	**2.9 (1.9)**	**10.2 (3.3)**	**2.6 (1.9)**	**3.5 (2.1)**	**15.2 (3.9)**	**3.0 (1.9)**	**5.0 (2.4)**	

M: Maggot **PP: Pre-pupa** **P: Pupa**

Figures in parentheses are square root (√x+1) transformation values

$CD_{0.05}$:

Maggots inoculated (M)	0.07
Intervals(I)	0.10
Population(P)	0.09
M x I x P	0.26

mean population of 1.9, 3.3 and 6.2 and all these values were significantly different from each other.

As evident from Table 25, the sciarids multiplied freely on the mycelium of *P. sajor caju* in all stages of cropping and initial population of two maggots each at spawning and pin head stage reached the mean level of 2.4/250 cc of substrate as compared to significantly high count of 6.8 at the initial inoculum of 200 insects. The insect population increased with progress in cropping period and the mean larval population of two, prepupal population of 0.1 and pupal population of 0.2 touched the respective stature of 15.2, 3.0 and 5.0 by the termination of experiment.

4.7.2. Effect on yield (Plate 17-19)

Statistically scrutinized data on treatment vise total sporocarp production of oyster mushroom when inoculated separately at spawning, pin head formation and both at spawning as well as pin head formation stage have been displayed in Tables 26-28.

Spawning time inoculations of 2, 20 and 200 maggots showed significantly adverse effect on total sporocarp production as compared to uninoculated control wherein 4353.3 g of fruiting bodies were produced. Minimum sporocarp production of 1823.3 g was obtained when 200 sciarid maggots were inoculated followed by 2833.3 and 3676.7 g yields achieved at respective inocula of 20 and 2 maggots. In all, yield losses to the tune of 58.1, 34.9 and 15.5 over control were assessed at inoculums levels of 2, 20 and 200 maggots. The data indicated that sporocarp production was density dependent i.e. yields reduced with corresponding increase in inocula. Weekly interpretation of data also showed a significant difference in the yield in the treatments receiving 20 or 20 maggots as compared to control for all the weeks (Table 26).

Table 26 Effect of different inoculation levels of *Sciara* maggots on sporophore yield of *P. sajor caju* (spawning time inoculation)

Maggots inoculated	Mean Sporophore yield (g)					Total yield (g)	Loss (%)
	1st week	2nd week	3rd week	4th week	5th week		
2	1146.7	1180.0	785.0	351.7	213.3	3676.7	15.5
20	1013.3	858.3	618.3	251.7	91.7	2833.3	34.9
200	778.3	513.3	363.3	106.7	61.7	1823.3	58.1
Control	1165.0	1330.0	940.0	533.3	385.0	4353.3	-
$CD_{(0.05)}$	**74.5**	**145.5**	**65.7**	**78.9**	**104.7**	**343.8**	

Control 2 larvae 20 larvae 200 larvae

Plate 17 Effect of sciarid larvae in *Pleurotus sajor caju* (spawning time inoculation)

Control 2 larvae 20 larvae 200 larvae

Plate 18 Effect of sciarid larvae in *Pleurotus sajor caju* (pin head formation time inoculation)

Control 2 larvae 20 larvae 200 larvae

Plate 19 Effect of sciarid larvae in *Pleurotus sajor caju* (spawning + pin head formation time inoculation)

The data regarding damaging potential of *Sciara* sp., when infesting the crop at pin head formation stage have been presented in Table 27. Highest sporocarp production of 4353.3g was obtained in untreated bags followed by 4205.0 g in bags infested with two maggots. Sporocarp production in these two treatments was statistically at par with each other indicating no adverse effect on the yields if small population of *Sciara* sp. infested the crop at late stage. However, significant decline in yields were recorded when bags were inoculated with 20 or 200 maggots wherein respective yields of 3808.3 and 3098.3 g were obtained. The yield losses increased with increase in pest density and reached the maximum of 28.8 per cent over control at highest level of insect inoculum. Weekly observation on yields indicated no significant difference of yield pattern at the lowest inoculums of two maggots with respect to uninoculated control. However, weekly pattern was disrupted at the higher level of treatments.

Table 27 Effect of different inoculation levels of *Sciara* maggots on sporophore yield of *P. sajor caju* (pin head formation time inoculation)

Maggots inoculated	Mean Sporophore yield (g)					Total yield (g)	Loss (%)
	1st week	2nd week	3rd week	4th week	5th week		
2	1121.7	1296.7	921.7	491.7	373.3	4205.0	3.41
20	1140.0	1015.0	910.0	441.7	301.7	3808.3	12.5
200	1096.7	913.3	630.0	288.3	170.0	3098.3	28.8
Control	1165.0	1330.0	940.0	533.3	385.0	4353.3	-
$CD_{(0.05)}$	**N.S.**	**71.6**	**64.4**	**55.7**	**98.6**	**375.8**	

Perusal of data unveiled in Table 28 indicated highest reduction in sporocarp production when larval populations infested the crop at an early stage of spawning followed by second inoculation at pin head formation. Under these conditions, even the lowest inoculum of two maggots incurred significant reduction in sporocarp production with respect to untreated healthy bags. Significantly reduced yields of 3291.7, 2345.0 and 1608.3 g of sporocarps were achieved at respective inocula of 2, 20 and 200 maggots as compared to the maximum of 4353.3 g attained in uninoculated bags. Yield losses to the tune of 24.4, 46.1 and 63.1 over control were assessed at the corresponding inoculum levels. Yield pattern for first two weeks was also disturbed in all the treatments except control.

Table 28 Effect of different inoculation levels of *Sciara* maggots on sporophore yield of *P. sajor caju* (spawning + pin head formation time inoculation)

Maggots inoculated	Mean Sporophore yield (g)					Total yield (g)	Loss (%)
	1st week	2nd week	3rd week	4th week	5th week		
2	1138.3	943.3	668.3	305.0	236.7	3291.7	24.4
20	850.0	741.7	466.7	198.3	88.3	2345.0	46.1
200	658.3	481.7	331.7	86.7	50.0	1608.3	63.1
Control	1165.0	1330.0	940.0	533.3	385.0	4353.3	-
$CD_{(0.05)}$	**71.9**	**49.9**	**53.5**	**34.9**	**79.3**	**96.2**	

4.7.3 Effect on flush pattern

Flush pattern in *P. sajor caju* is more or less similar to *A. bisporus* in which sporocarps emerge in flushes at weekly gaps. However, the bags having insect inoculations showed disruption in normal flush pattern as evident from Figures 11-13.

A normal flush pattern of comparatively low sporocarp emergence in first week of cropping followed by higher yields in second week was observed when bags were infested with minimum number of two maggots at spawning or pin head formation stage (Figure 11). Thereafter, the yields declined progressively with time until the cropping ceased. However, when similar inoculum infested the crop once at spawning and then at pin head formation stage, though, the yields were at par with other treatments in the first week, a drastic decline occurred in second week.

Thereafter, a progressive decline in sporocarps emergence was recorded but interestingly by fifth week the fruiting body production in this treatment was at par with that of spawning time treatment of same inoculum.

As depicted in Figure 12, disruption in flush pattern was observed in all the treatments receiving 20 maggots irrespective of the cropping stage at which infestation occurred. In contrary, the untreated bags produced slightly lower yields in first week followed by better yields in second week. Thereafter, a steady decline was seen in all the treatments.

Data assembled in Figure 13, indicated almost similar sporocarp production in untreated bags and bags inoculated with 200 maggots at pin heads formation stage. A significant difference in pattern occurred thereafter. While, uninoculated bags registered an increase in sporocarp yields during second week, all the insect inoculated bags yielded poor sporocarp production at this period. However, beyond this period a progressive decline in sporocarp emergence occurred in all the treatments.

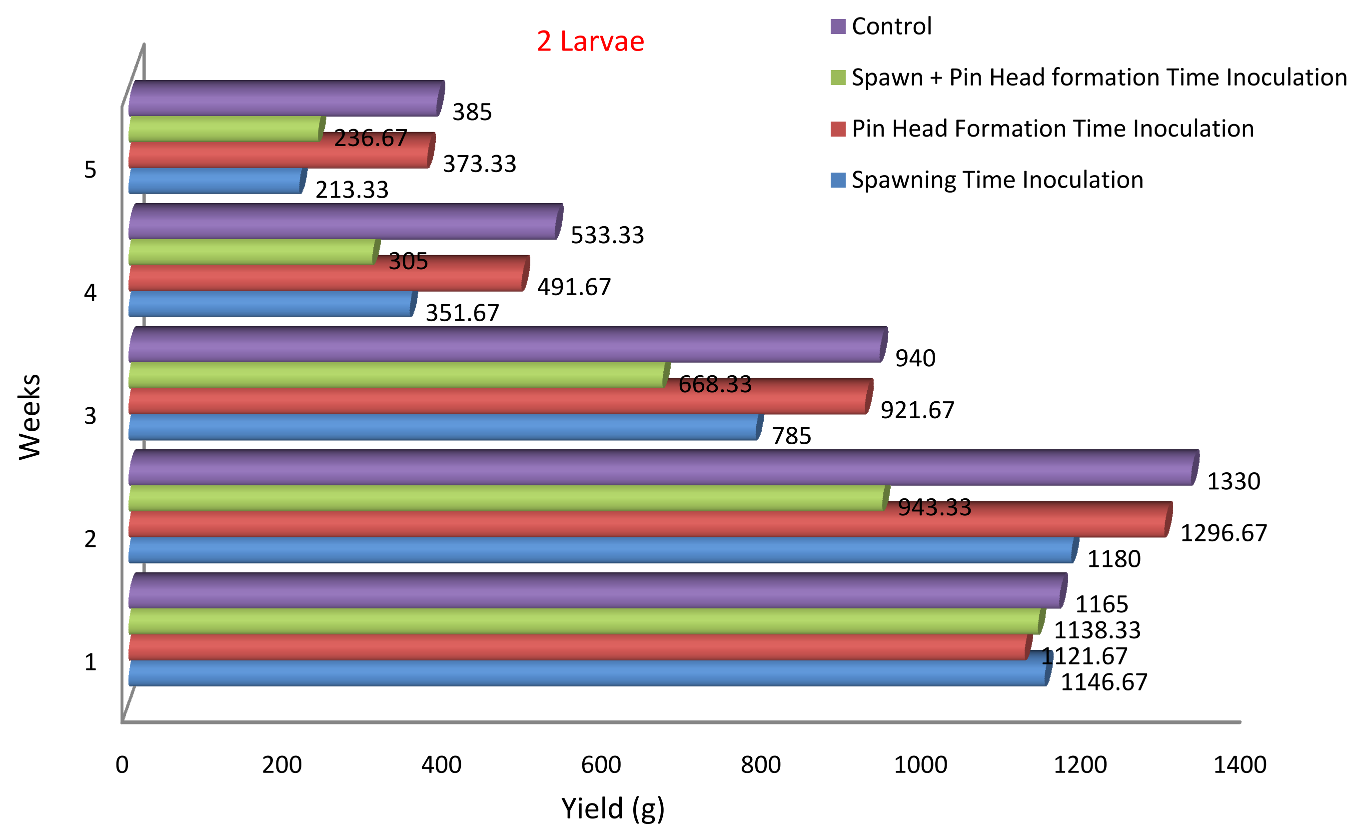


Fig 11 Effect of different time inoculation of two sciarid larvae on flush pattern of *P. sajor caju*

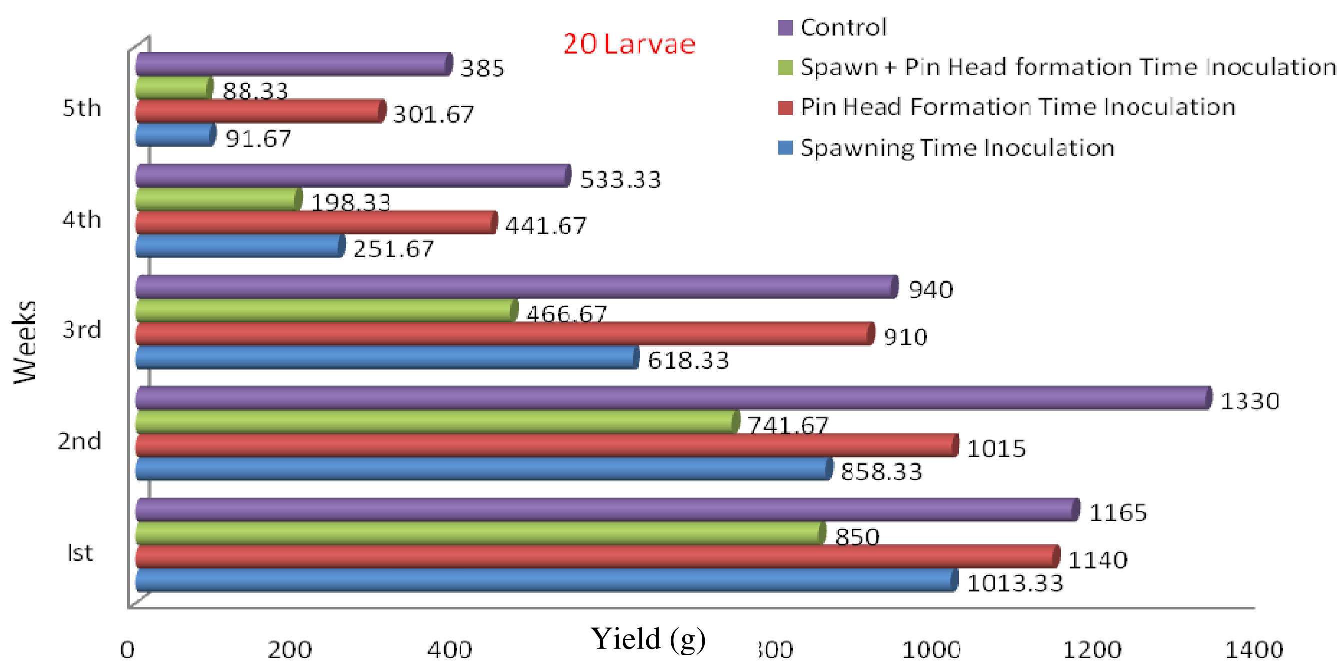


Fig 12 Effect of different time inoculations of 20 sciarid larvae on flush pattern of *P. sajor caju*

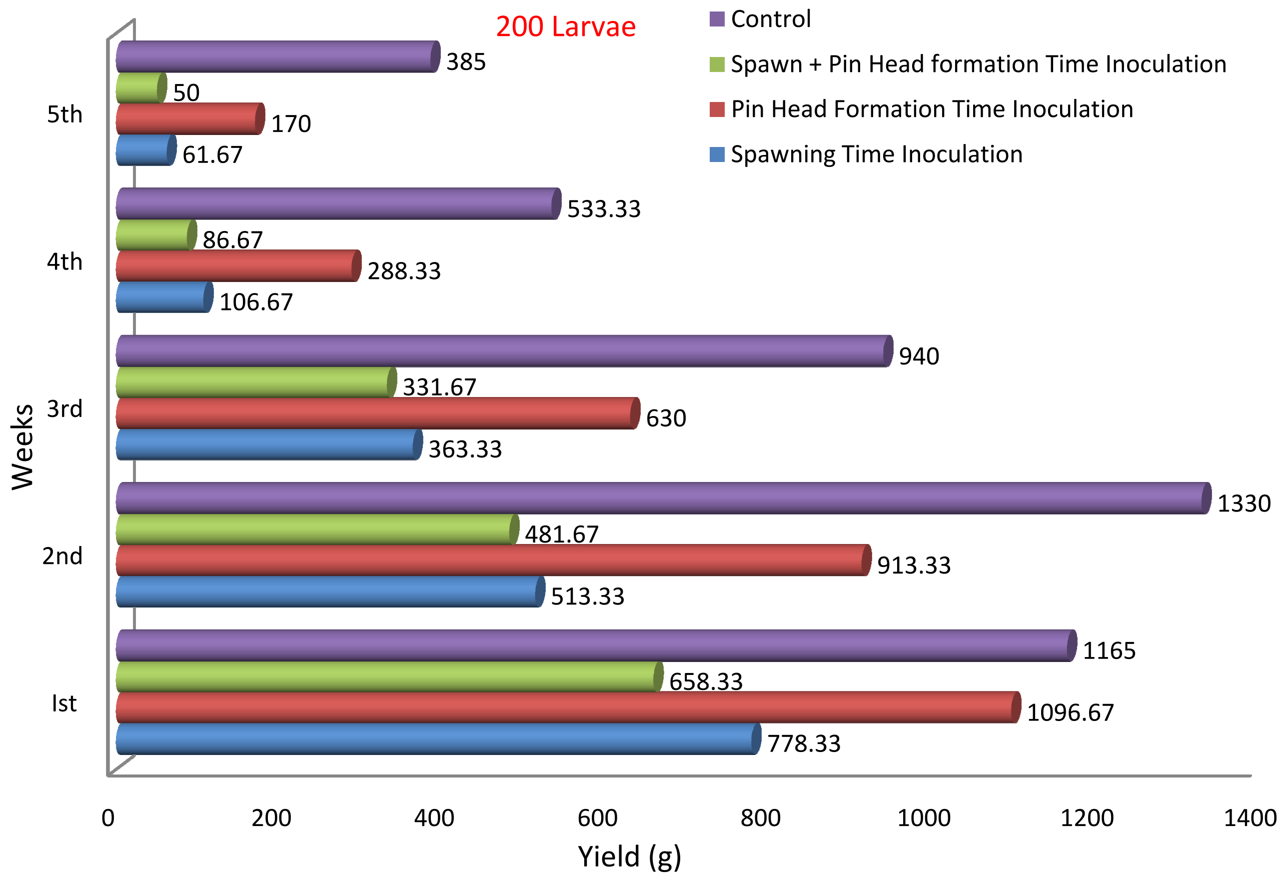


Fig 13 Effect of different time inoculations of 200 sciarid larvae on flush pattern of *P. sajor caju*

Correlation expression depicted in Table 29 signified a negative relationship between the larval population and sporocarps yields in oyster mushroom irrespective of the stage of cropping at which infestation occurred. It meant higher the insect population, poorer was the sporocarps emergence and vice versa.

Table 29 Correlation between sciarid population and sporocarp yield of *P. sajor caju*

	Yield	Larval Population at spawning	Larval Population at pin head formation	Larval Population at spawning + pin head formation
Yield	1.0000			
Larval Population at spawning	-0.9857*	1.0000		
Larval Population at pin head formation	-0.9754*	0.9816*	1.0000	
Larval Population at spawning + pin head formation	-0.9906*	0.9934*	0.9938*	1.0000

*Significant at 5 % level of confidence

The emerged sporocarps were observed for visual damage if any and were quantified against the healthy sporocarps at different levels of insect inocula as well as stage of inoculation in oyster mushrooms. The data have been assembled in Table 30.

Table 30 Effect of different inocula of *Sciara* maggots on morphology of *P. sajor caju* sporocarps

Maggots inoculated	Healthy sporophores (%)			Mean
	Spawning time inoculation	Pin Head time inoculation	Spawning + Pin Head time inoculation	
2	89.2 (70.8)	90.9 (72.6)	84.8 (67.0)	88.3 (70.1)
20	84.6 (66.9)	89.2 (70.8)	82.7 (65.4)	87.8 (67.7)
200	83.1 (65.7)	85.1 (67.3)	77.3 (61.5)	81.8 (64.8)
Control	99.3 (86.1)	99.3 (86.1)	99.3 (86.1)	99.3 (86.1)
Mean	89.0 (72.4)	91.2 (74.2)	86.0 (70.4)	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Inoculation time(I) 1.5 Maggots (M) 1.7 I x M 2.9

Near complete healthy crop (99.3%) was obtained in uninoculated bags wherein hardly any sporocarp wore an unhealthy look. It was observed that as the inoculum level increased, a decrease in the per cent healthy sporocarps occurred. The mean count of healthy sporocarps declined from 88.3 per cent at inoculum level of two maggots to 87.8 per cent at 20 maggots and 81.8 per cent when 200 maggots were inoculated, all values being statistically different from each other.

Later the cropping stage at time of insect infestation, higher was the per cent healthy crop. Crop to the tune of 91.2 per cent was healthy when infestation occurred at pin head formation stage in comparison to 89.0 per cent when infestation occurred at spawning stage. Maximum damage was observed when insect attacked the crop at spawning as well as pin head formation stage; damage increasing with increase in inoculum level. Only 77.3 per cent crop remained healthy at inoculum level of 200 maggots elevated to 82.7 and 84.8 per cent at respective inocula of 20 and two maggots.

Visual symptoms were spectacular in oyster mushrooms where infested pin heads became soggy due to soaking of water and did not develop into fruiting bodies. Morphological distortion of sporocarp was evident in the form of mushy appearance and visible tunneling of stipe by the pest. Frequently, the maggots were spotted feeding on the gills. The infested sporocarps had a tendency to drupe down.

4.8 Biomanagement of *Sciara* sp. in *A. bisporus* and *P. sajor caju*

Mushroom cultivation is a unique biological system in which crop is grown in the enclosed chambers and where fruiting bodies emerge in succession of flushes within a single crop of six to eight weeks duration. Once spawned, the growers are reluctant to disturb the bags/beds till the cessation of crop. Thus the crop, though, highly susceptible to insect pests, management thereof becomes a challenge. Use of chemical insecticides is not advisable as fruiting bodies are very delicate, soft with a highly absorbing texture and are usually consumed fresh. Pest management practices in this crop thus need to be crop friendly. Since, Neem (*Azadirachta indica* A. Juss) is known for its insecticidal properties, two Neem products viz. Neem Seed Kernel Extract (NSKE) and Neem Seed Kernel Powder (NSKP) were tried against the test insect. In addition, two entomopathogenic nematodes viz., *Steinernema carpocapsae* and *Heterorhabditis indica* were inoculated to test their efficacy against *Sciara* maggots. Decision to test these bioagents was made on the basis of their outcome against some other insect pests in mushrooms as well as some other crops.

4.8.1 Management of *Sciara* sp. in *A. bisporus*

The experiment was conducted in two phases.

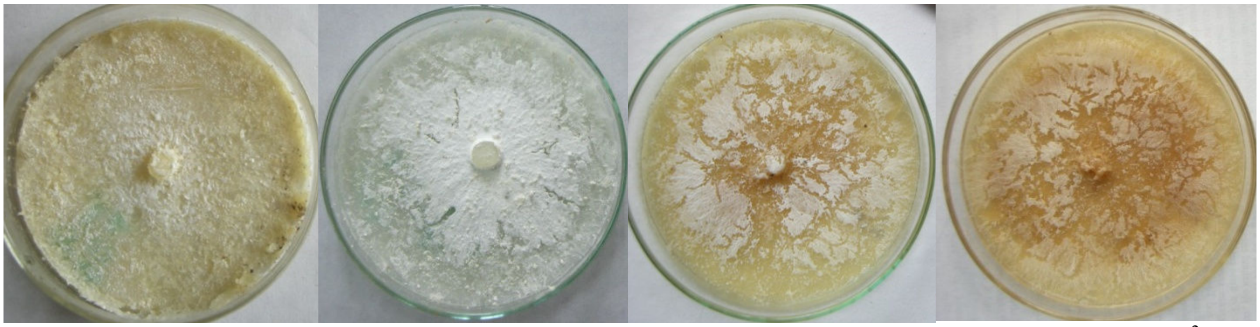
In vitro

Petri plates of 9cm diameter with fully impregnated mycelium of *A. bisporus* either on NSKE/NSKP treated malt agar extract medium or medium free of these products as per the requirement of specific treatment were used for the purpose. Observations regarding treatment vise mycelial depletion and larval count were recorded at 2, 4, 6, 8 and 10 days of treatment.

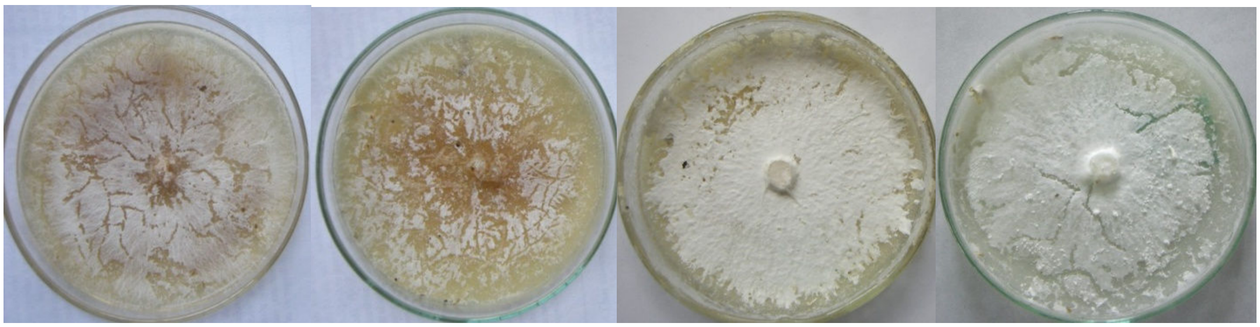
4.8.1.1 Effect on mycelial growth (Plate 20)

Data referred in Table 31 pin points the efficacy of Neem as well as entomophagous nematodes when used alone or in combination against the test insect. Mycelial depletion increased with time in all the treatments. Mean depreciation of 11.3 per cent at two days reached the mean value of 30.8 per cent by ten days. Best results were obtained in T10 (NSKE + *H. indica* @10^3 IJs) that showed minimum mean depreciation of 10.1 per cent mycelium which was significantly less that 12.3 per cent achieved in T8 (NSKE + *S. carpocapsae* @10^3IJs). This was closely followed by T6 and T14 wherein per cent of mycelial diminishing were 13.6 and 13.4, respectively. Untreated insect inoculated control showed maximum mycelial depreciation that reached from 29.9 per cent at two days to 89.9 per cent by tenth day. All the treatments showed significant efficacy at all the time of observations with respect to control. The most effective was found to be T10 wherein minimum mycelial damage was observed at all the intervals of observation. The mycelial depreciation of 5.3 per cent at two days reached merely 14.8 per cent at ten days. This was followed by T8 (NSKE + *S. carpocapsae* @10^3IJs) and T6 (*H. indica* @ 10^3IJs) in which respective mycelial depreciations of 17.2 and 19.2 were recorded at ten days, both being statistically at par.

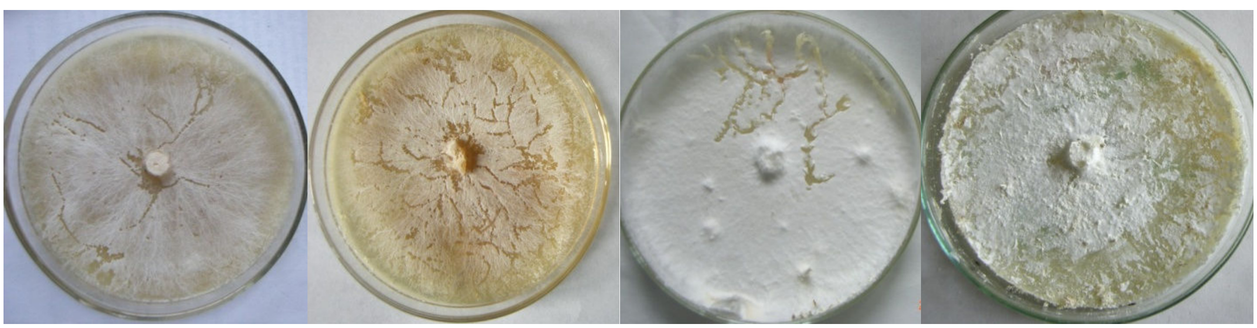
The interpretations from these data signify the efficacy of all the treatments on the mycelial growth of *A. bisporus* in presence of *Sciara* maggots. None of the treatments seemed to impair the mycelial growth of *A. bisporus* in Petri plates.



Control NSKE (1%) NSKP (1%) *S. carpocapsae* (10^2 IJs)



S. carpocapsae (10^3IJs) *H. indica* (10^2 IJs) *H. indica* (10^3IJs) NSKE+ *S. carpocapsae* (10^2IJs)



NSKE+ *S. carpocapsae* (10^3IJs) NSKE+ *H. indica* (10^2IJs) NSKE+ *H. indica* 10^3IJs) NSKP+ *S. carpocapsae* (10^2IJs)

NSKP+ *S. carpocapsae* (10^3IJs) NSKP+ *H.indica* (10^2IJs) NSKP+ *H.indica* (10^3IJs)

Plate20: Effect of Neem product and entomopathogenic nematodes on *Sciara* sp. affecting the mycelial growth of *Agaricus bisporus* (*in vitro*)

Table 31 Effect of Neem products and entomopathogenic nematodes on *Sciara* sp. affecting the mycelial growth of *A. bisporus* (*in vitro*)

Treatment	Per cent mycelial depletion (days)					Mean
	2	4	6	8	10	
T1 (NSKE 1.0%)	9.7 (18.1)	20.6 (26.9)	27.4 (31.5)	30.8 (33.7)	30.7 (33.6)	**23.5 (28.5)**
T2 (NSKP 1.0%)	14.3 (22.2)	27.6 (31.7)	38.6 (38.4)	42.9 (40.8)	45.3 (42.3)	**33.7 (35.1)**
T3 (*S. carpocapsae* 1 x 10^2)	16.4 (23.8)	27.0 (31.3)	39.4 (38.4)	42.9 (40.9)	46.1 (42.3)	**34.4 (35.5)**
T4 (*S. carpocapsae* 1 x 10^3)	9.9 (18.4)	12.8 (20.9)	17.7 (24.8)	20.9 (27.2)	23.6 (29.1)	**17.0 (24.1)**
T5 (*H. indica* 1 x 10^2)	12.9 (21.1)	20.8 (27.1)	24.6 (29.7)	28.0 (31.9)	30.6 (33.6)	**23.4 (28.7)**
T6 (*H. indica* 1 x 10^3)	7.2 (15.5)	11.3 (19.6)	14.3 (22.2)	16.0 (23.6)	19.2 (25.9)	**13.6 (21.4)**
T7 (NSKE + *S. carpocapsae* 1 x 10^2)	10.3 (18.6)	14.4 (22.3)	21.6 (27.7)	23.5 (28.9)	25.8 (30.5)	**19.1 (25.6)**
T8 (NSKE + *S. carpocapsae* 1 x 10^3)	5.3 (13.3)	10.9 (19.2)	13.2 (21.3)	15.0 (22.8)	17.2 (24.5)	**12.3 (20.2)**
T9 (NSKE + *H. indica* 1 x 10^2)	7.3 (15.6)	13.7 (21.7)	19.4 (26.1)	21.4 (27.5)	24.6 (29.7)	**17.3 (24.1)**
T10 (NSKE + *H. indica* 1 x 10^3)	5.3 (13.3)	7.9 (16.2)	10.6 (19.0)	12.0 (20.2)	14.8 (22.6)	**10.1 (18.3)**
T11 (NSKP + *S. carpocapsae* 1 x 10^2)	12.1 (20.3)	18.7 (25.6)	24.8 (29.9)	27.3 (31.5)	30.8 (33.7)	**23.7 (28.2)**
T12 (NSKP + *S. carpocapsae* 1 x 10^3)	10.5 (18.8)	14.2 (22.1)	17.1 (24.4)	18.4 (25.4)	20.5 (26.3)	**16.1 (23.5)**
T13 (NSKP + *H. indica* 1 x 10^2)	12.1 (20.3)	18.4 (25.4)	21.2 (27.4)	22.8 (28.5)	24.4 (29.6)	**19.8 (26.2)**
T14 (NSKP + *H. indica* 1 x 10^3)	6.5 (14.8)	10.1 (18.5)	14.8 (22.6)	16.6 (24.0)	18.8 (25.7)	**13.4 (21.1)**
T15 (Untreated Control)	29.9 (33.1)	45.9 (42.6)	57.8 (49.5)	80.3 (63.7)	89.9 (71.5)	**60.7 (52.1)**
Mean	**11.3 (19.2)**	**18.3 (25.5)**	**24.2 (29.9)**	**27.8 (32.7)**	**30.8 (35.8)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Time interval (I) 0.42
Treatments (T) 0.73
I x T 1.63

4.8.1.2 Effect on larval population of *Sciara* sp.

Larval population retrieved from various treatments at different time intervals of 2, 4, 6, 8 and 10 days of inoculation (Table 32) displayed significantly high mortality in all the treatments as compared to control. The mean per cent mortality increased from 35.4 per cent at two days to 64.9 at 10 day. Similarly, the mean larval mortality of 3.7 per cent in untreated control due to natural reasons was much lower than any of the treatment. The least mean larval

Table 32 Effect of Neem product and entomopathogenic nematodes on population of *Sciara* sp. affecting *A. bisporus* (*in vitro*)

Treatment	Larval mortality (%) at					Mean
	2	4	6	8	10	
T1 (NSKE 1.0%)	33.3 (35.2)	46.7 (43.1)	53.3 (46.9)	56.7 (48.8)	58.3 (49.8)	**49.7 (44.7)**
T2 (NSKP 1.0%)	18.3 (25.2)	30.0 (33.1)	35.0 (36.1)	40.0 (39.2)	46.7 (43.1)	**34.0 (35.3)**
T3 (*S. carpocapsae* 1 x 10^2)	13.3 (21.3)	23.3 (28.8)	33.3 (35.2)	38.3 (38.2)	55.0 (47.9)	**32.7 (34.3)**
T4 (*S. carpocapsae* 1 x 10^3)	26.7 (31.1)	53.3 (46.9)	56.7 (48.9)	61.7 (51.8)	63.3 (52.9)	**52.3 (46.3)**
T5 (*H. indica* 1 x 10^2)	23.3 (28.9)	38.3 (38.0)	51.7 (45.9)	60.0 (50.8)	66.7 (54.7)	**48.0 (43.7)**
T6 (*H. indica* 1 x 10^3)	48.3 (44.0)	61.7 (51.8)	66.7 (54.9)	73.3 (58.9)	78.3 (62.2)	**65.7 (54.4)**
T7 (NSKE + *S. carpocapsae* 1 x 10^2)	38.3 (38.2)	48.3 (44.0)	58.3 (49.9)	65.0 (53.8)	71.7 (57.9)	**56.3 (48.8)**
T8 (NSKE + *S. carpocapsae* 1 x 10^3)	55.0 (47.9)	63.3 (52.9)	68.3 (55.7)	73.3 (59.0)	78.3 (62.3)	**67.7 (55.6)**
T9 (NSKE + *H. indica* 1 x 10^2)	43.3 (41.2)	58.3 (49.9)	70.0 (56.8)	73.3 (58.9)	76.7 (61.2)	**64.3 (53.6)**
T10 (NSKE + *H. indica* 1 x 10^3)	53.3 (46.9)	66.7 (54.8)	71.7 (57.8)	76.7 (61.1)	83.3 (65.9)	**70.3 (57.3)**
T11 (NSKP + *S. carpocapsae* 1 x 10^2)	36.7 (37.1)	48.3 (44.0)	55.0 (47.9)	61.7 (51.7)	68.3 (55.8)	**54.0 (47.3)**
T12 (NSKP + *S. carpocapsae* 1 x 10^3)	41.7 (40.2)	56.7 (48.8)	61.7 (51.8)	66.7 (54.7)	73.3 (58.9)	**60.0 (50.7)**
T13 (NSKP + *H. indica* 1 x 10^2)	46.7 (43.1)	51.7 (45.9)	66.7 (54.8)	70.0 (56.8)	73.3 (59.0)	**61.7 (51.9)**
T14 (NSKP + *H. indica* 1 x 10^3)	51.7 (45.9)	58.3 (49.9)	65.0 (53.8)	71.7 (59.0)	75.0 (65.5)	**64.3 (54.2)**
T15 (Untreated Control)	1.7 (4.3)	3.3 (8.6)	3.3 (8.6)	5.0 (12.9)	5.0 (12.9)	**3.7 (9.5)**
Mean	**35.4 (35.3)**	**47.2 (42.7)**	**54.4 (47.0)**	**59.6 (50.3)**	**64.9 (53.6)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Time interval (I) 1.72

Treatments (T) 2.98 I x T N.S.

kill of 32.7 per cent in T3 (*S. carpocapsae* @ 10^2 IJs) was appreciably higher than control. All other treatments produced significantly higher insect mortality. The best results were obtained in T10 (NSKE + *H. indica* @10^3 IJs) where 53.3 per cent maggots were killed just at two days that increased to 66.7, 71.7, 76.7 and 83.3 per cent at 4, 6, 8 and 10 days, respectively. Similar treatment but with 10^2 *H. indica* (T9) caused 76.7 per cent insect mortality. Among entomophagous nematodes, *H. indica* when inoculated alone caused significantly higher mean insect kill than *S. carpocapsae* at both the levels of inoculum. Among Neem products, NSKE (T1) produced significantly higher mean mortality (49.7 %) than NSKP that killed 34.0 per cent maggots. Nematodes were in no was affected by the application of Neem products during medium preparation and both in combination caused appreciably better results than when applied alone as evident from mortality results perceived in treatments T7 to T14.

4.8.1.3 Correlation between larval mortality and damaging potential of sciarid

Correlation expressions depicted in Table 33 signified a negative relationship between the larval mortality and mycelial depletion in button mushrooms. It meant that more the number of maggots killed, lesser was the mycelial depreciation. Naturally, the higher insect population would cause the greater mycelial damage and vice versa.

Table 33 Correlation between larval mortality and damaging potential of sciarid

	Mycelial Depletion	**Larval Mortality**
Mycelial Depletion	1.000	
Larval Mortality	-0.94*	1.000

*Significant at 5 % level of confidence

In vivo

Studies were conducted in 1 kg compost bags inoculated as per the required treatments. Observations regarding mycelial growth and sporophore yield were recorded.

4.8.2 Effect on mycelial growth (Spawning time inoculation)

Insects when inoculated at spawning, fed upon mushroom mycelium, causing its appreciable depreciation by the time of casing as evident from observations recorded in T15 (untreated insect inoculated control) wherein 80.5 per cent mycelial growth was recorded as compared to 96.2 per cent in T16 (untreated uninoculated control) at casing time. All the treatments barring T2, T11, T12, T13 and T14 resulted into significantly more mean mycelial growth as compared to untreated insect inoculated control (T15). Highest mean mycelial growth of 54.9 per cent recorded in T6 was statistically at par with T1, T5, T7, T8, T9 and T10 indicating these treatments to be effective against the test insect. However, mean mycelial growth in none of the treatments was statistically equal to T16 (57.5 %). This meant that though effective with respect to untreated inoculated control (T15), these treatments could not improve the mycelial growth to the level of untreated uninoculated control (T16). Mean mycelial growth of 86.5 per cent at casing reduced significantly to 7.6 at 45 days of casing as mycelium was exhausted partly due to sporophore production and partly due to feeding by the insect maggots (Table 34).

4.8.2.1 Effect on mycelial growth of *A. bisporus* (Spawning + Casing time inoculation)

Data placed in Table 35 showed non significant variations in mycelial growth both at casing and at 45 days of casing in various treatments. Significantly poor mean mycelial spread of 40.7 per cent recorded in untreated insect inoculated control was at par with 44.0 per cent in T2 receiving NSKP during spawning indicating NSKP to be ineffective in relation to mycelial growth. Among the treatments receiving one or the other bioagents, highest mean mycelial spread of 51.8 per cent observed in T10 was at par with T4, T5, T6, T7, T8, T9, T11, T12, T13 and T14 indicating all these treatments to be equally effective in concern to their effect on mean mycelial growth. However, mean mycelial growth in none of the applications was at par with T16 (untreated uninoculated control).

4.8.3. Effect on yield of *A. bisporus* (Spawning time inoculation)

Efficacy of Neem products viz. NSKE and NSKP and entomophagous nematodes viz., *S. carpocapsae* and *H. indica* each at two inocula of 10^4 and 10^5 IJs was assessed in terms of the effect of *Sciara* sp. on the yield of *A. bisporus* in presence of these bioagents and has been arranged in Table 36.

Sciara maggots inflicted heavy damage to mushroom crop when infestation occurred at an early stage of spawning. Sporophore yields declined significantly from 196.7 g in T16 (untreated uninoculated control) to 149.0 g in T15 (untreated inoculated control) causing a loss of 24.3 per cent. Out of number of treatments involving biocontrol agents to combat the effect of these pests, the best results were obtained in T6, T5 and T10 receiving lone application of 10^5 IJs of *H. indica*, 10^4 IJs of *H. indica* and NSKE along with 10^5 IJs of *H. indica*, respectively. Button production of 189.7, 175.3 and 175.0 g achieved in these treatments was at par with 196.7 g attained in untreated uninoculated control. Solo application of NSKE in the compost (T1) yielded

Table 34 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the mycelial growth of *A. bisporus* (spawning time inoculation)

Treatment	Mycelial growth (%)		Mean
	Casing	45 days of casing	
T1 (NSKE @ 1.0%)	88.1 (69.9)	8.3 (16.6)	**48.2 (43.3)**
T2 (NSKP @ 1.0%)	80.0 (63.6)	4.9 (12.5)	**42.5 (38.1)**
T3 (*S. carpocapsae* @ 1 x 10^4)	82.4 (65.4)	6.1 (14.2)	**44.3 (39.8)**
T4 (*S. carpocapsae* @1 x 10^5)	84.9 (67.2)	8.2 (16.6)	**46.6 (41.9)**
T5 (*H. indica* @1 x 10^4)	89.4 (71.3)	7.1 (15.4)	**48.3 (43.4)**
T6 (*H. indica* @1 x 10^5)	95.8 (78.5)	14.2 (22.0)	**54.9 (50.3)**
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	85.3 (67.6)	6.3 (14.4)	**45.8 (41.0)**
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	87.6 (69.5)	7.9 (16.3)	**47.80 (42.9)**
T9 (NSKE + *H. indica* @ 1 x 10^4)	88.2 (70.1)	8.3 (16.6)	**48.3 (43.4)**
T10 (NSKE + *H. indica* @ 1 x 10^5)	93.2 (75.5)	9.1 (17.5)	**54.6 (46.5)**
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	80.0 (63.6)	3.0 (9.8)	**41.5 (36.7)**
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	82.9 (65.8)	3.9 (11.2)	**43.4 (38.5)**
T13 (NSKP + *H. indica* @ 1 x 10^4)	81.6 (64.8)	4.7 (12.2)	**43.2 (38.5)**
T14 (NSKP + *H. indica* @ 1 x 10^5)	88.0 (69.9)	6.2 (14.4)	**47.1 (42.2)**
T15 (Untreated inoculated control)	80.5 (63.9)	3.9 (11.4)	**42.2 (37.6)**
T16 (untreated uninoculated control)	96.2 (79.9)	19.9 (26.4)	**58.0 (53.2)**
Mean	**86.5 (69.2)**	**7.6 (15.5)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$	Time interval (I)	1.43
	Treatments (T)	4.05
	I x T	N.S.

173.3 g sporophores. This yield was quantitatively higher than the yield achieved in T15 as far as statistic is concerned, thus, making this treatment also to be effective against the pest. However, this yield was significantly lower than that achieved in T5, T6 and T10. The quantum of fruiting bodies

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produced in all other treatments was statistically at par with the yield of 149.0 g recorded in T15, thus making them ineffective as far as their effect on *Sciara* population affecting the yield of *A. bisporus* is concerned.

Table 35 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the mycelial growth of *A. bisporus* (Spawning+casing time inoculations)

Treatment	Mycelial growth (%)		Mean
	Casing	45 days of casing	
T1 (NSKE @ 1.0%)	91.4 (69.1)	7.2 (16.6)	**45.9 (42.9)**
T2 (NSKP @ 1.0%)	84.2 (66.9)	3.8 (15.1)	**44.0 (40.9)**
T3 (*S. carpocapsae* @ 1 x 10^4)	85.8 (68.1)	4.8 (15.5)	**45.3 (41.8)**
T4 (*S. carpocapsae* @1 x 10^5)	88.0 (69.9)	5.5 (21.7)	**46.8 (45.8)**
T5 (*H. indica* @1 x 10^4)	88.8 (70.8)	6.6 (15.9)	**47.2 (44.2)**
T6 (*H. indica* @1 x 10^5)	95.0 (72.5)	9.4 (18.7)	**49.8 (44.1)**
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	80.5 (72.1)	3.4 (16.2)	**46.8 (44.4)**
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	83.5 (73.3)	3.6 (16.9)	**47.3 (45.1)**
T9 (NSKE + *H. indica* @ 1 x 10^4)	85.2 (73.6)	4.2 (17.1)	**47.9 (45.4)**
T10 (NSKE + *H. indica* @ 1 x 10^5)	86.6 (77.9)	6.6 (18.1)	**51.8 (47.6)**
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	81.0 (67.4)	2.2 (19.8)	**43.6 (43.5)**
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	83.0 (71.3)	3.6 (18.4)	**47.2 (44.9)**
T13 (NSKP + *H. indica* @ 1 x 10^4)	81.4 (71.4)	4.8 (19.3)	**47.2 (45.3)**
T14 (NSKP + *H. indica* @ 1 x 10^5)	83.9 (71.6)	5.0 (20.9)	**47.5 (46.2)**
T15 (Untreated inoculated control)	80.5 (63.9)	0.9 (10.9)	**40.7 (37.4)**
T16 (untreated uninoculated control)	98.2 (83.9)	14.5 (26.4)	**56.4 (55.1)**
Mean	**89.1 (71.5)**	**5.0 (17.6)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Time interval (I) 1.48

Treatments (T) 4.34 I x T N.S.

Yield losses reduced to just 3.6 per cent when 10^5 IJs of *H. indica* were inoculated at spawning time. This was followed by 10.9, 11.03 and 11.9 per cent losses in T5, T10 and T1, respectively, receiving corresponding applications of 10^4 IJs of *H. indica*, NSKE + 10^5 IJs of *H. indica* and single application of NSKE. Sporophore losses in other treatments were appreciably high, varying from 15.6 per cent in T4 to 23.7 per cent in T11. Losses in these treatments were at par with 24.3 per cent loss recorded in untreated inoculated control (T15).

Table 36 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the yield of *A. bisporus* (Spawning time inoculation)

Treatment	Yield per bag (g)	Yield loss (%)
T1 (NSKE @ 1.0%)	173.3	11.9
T2 (NSKP @ 1.0%)	150.3	23.6
T3 (*S. carpocapsae* @ 1 x 10^4)	162.7	17.3
T4 (*S. carpocapsae* @1 x 10^5)	166.0	15.6
T5 (*H. indica* @1 x 10^4)	175.3	10.9
T6 (*H. indica* @1 x 10^5)	189.7	3.6
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	160.0	18.7
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	164.0	16.6
T9 (NSKE + *H. indica* @ 1 x 10^4)	162.7	17.3
T10 (NSKE + *H. indica* @ 1 x 10^5)	175.0	11.0
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	150.0	23.7
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	156.7	20.4
T13 (NSKP + *H. indica* @ 1 x 10^4)	155.0	21.2
T14 (NSKP + *H. indica* @ 1 x 10^5)	156.7	20.4
T15 (Untreated inoculated control)	149.0	24.3
T16 (untreated uninoculated control)	196.7	-
C.D.	**24.46**	-

4.8.3.1 Effect on yield of *A. bisporus* (Casing time inoculation)

The effect of *Sciara* sp. on sporophore production of *A. bisporus* in presence/absence of different biomanagement applications when insect infested the crop at casing time has been given in Table 37.

Table 37 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the yield of *A. bisporus* (Casing time inoculation)

Treatment	Yield per bag (g)	Yield loss (%)
T1 (NSKE @ 1.0%)	178.0	5.3
T2 (NSKP @ 1.0%)	165.0	12.2
T3 (*S. carpocapsae* @ 1 x 10^4)	169.7	9.8
T4 (*S. carpocapsae* @1 x 10^5)	165.7	11.9
T5 (*H. indica* @1 x 10^4)	175.7	6.6
T6 (*H. indica* @1 x 10^5)	181.7	3.3
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	172.0	8.5
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	176.3	6.2
T9 (NSKE + *H. indica* @ 1 x 10^4)	174.3	7.3
T10 (NSKE + *H. indica* @ 1 x 10^5)	177.7	5.5
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	163.7	12.9
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	168.0	10.6
T13 (NSKP + *H. indica* @ 1 x 10^4)	167.7	10.8
T14 (NSKP + *H. indica* @ 1 x 10^5)	170.0	9.6
T15 (Untreated inoculated control)	151.7	19.3
T16 (untreated uninoculated control)	188.0	-
C.D.	**N.S.**	-

Test insect when inoculated at a later stage in cropping (casing time) was not able to incur significant quantitative damage as far as button production is concerned. Button production in insect inoculated control was 151.7 g as compared to the yields of 188.0 g achieved in insect free control (T16). Sporophore yields in other treatments receiving one or the other bioagents varied from minimum of 163.7 in T11 receiving NSKP + 10^4 IJs *S. carpocapsae* to maximum of 180.7 g in T6 receiving lone inoculum of 10^5 IJs of *H. indica*. This variation in yields was non significant. Yield reduction in insect inoculated control was 19.3 as compared to reduction range of 3.3 to12.9 per cent in treated applications.

4.8.3.2 Effect on yield of *A. bisporus* (Spawning + Casing time inoculation)

The observations concerning sporophore production of *A. bisporus* when *Sciara* sp. was inoculated at spawning followed by second infestation at casing time have been presented in Table 38.

The test insect incurred heavy quantitative damage to the mushroom sporophores as its maggots fed voraciously on growing mycelium as well as buttons. The sporophore production was reduced to mere 96.7 g in insect inoculated control (T15) as compared to 202.7 g in insect free control (T16). Though, quantitative sporophore production increased significantly in all the treatments when compared to T15, it was not at par with T16 in any of the treatment. Among the treatments receiving Neem products and/or entomophagous nematodes highest yield of 176.3 g was attained in T6 receiving 10^5 IJs of *H. indica* which was significantly similar to 165.3 in T5 (10^4 IJs of *H. indica*) and 162.0 g in T1 (NSKE @1%). These results signified lone applications of *H. indica* or NSKE to be the most effective against *Sciara* maggots in turn improving the sporophore yields when dual infestation of the insect occurred at spawning as well as casing stage. Yield losses to the tune of 52.3 per cent assessed in T15 (untreated inoculated control) were appreciably higher than the bags receiving biocontrol agents. Minimum yield losses of 13.0 per cent were recorded in T6 closely followed by 18.4 and 20.1 per cent in respective treatments of T5 and T1. Yield losses varying from 24.2 to 37.5 per cent in other treatments were substantially high.

Table 38 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the yield of *A. bisporus* (Spawning + Casing time inoculation)

Treatment	Yield per bag (g)	Yield loss (%)
T1 (NSKE @ 1.0%)	162.0	20.1
T2 (NSKP @ 1.0%)	136.0	32.9
T3 (*S. carpocapsae* @ 1 x 10^4)	141.0	30.4
T4 (*S. carpocapsae* @1 x 10^5)	150.0	26.0
T5 (*H. indica* @1 x 10^4)	165.3	18.4
T6 (*H. indica* @1 x 10^5)	176.3	13.0
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	148.3	26.8
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	145.0	28.5
T9 (NSKE + *H. indica* @ 1 x 10^4)	140.3	30.8
T10 (NSKE + *H. indica* @ 1 x 10^5)	153.7	24.2
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	119.3	41.1
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	126.7	37.5
T13 (NSKP + *H. indica* @ 1 x 10^4)	131.0	35.4
T14 (NSKP + *H. indica* @ 1 x 10^5)	141.0	30.4
T15 (Untreated inoculated control)	96.7	52.3
T16 (untreated uninoculated control)	202.7	-
C.D.	**20.19**	-

4.8.4 Correlation studies

Data regarding correlation between mycelial growth and sporophore yield placed in Table 39 infers a highly positive relationship between the two. Higher the rate of mycelial impregnation more was the quantitative button production.

Table 39 Correlation between yield and mycelial growth in *A. bisporus*

	Yield	Mycelial growth in spawning time treatment	Mycelial growth in spawning + casing time treatment
Yield	1.000		
Mycelial growth in spawning time treatment	0.9788	1.000	
Mycelial growth in spawning + casing time treatment	0.9719	0.9818	1.000

*Significant at 5 % level of confidence

4.8.5 Management of *Sciara* sp. in *P. sajor caju*

The experiment was conducted in two phases.

In vitro

Petri plates of 9cm diameter with fully impregnated mycelium of *P. sajor caju* either on NSKE/NSKP treated potato dextrose agar medium or medium free of these products as per the requirement of specific treatment were used for the purpose. Observations regarding treatment vise mycelial depletion and larval count were recorded at 2, 4, 6, 8 and 10 days of treatment.

4.8.5.1 Effect on mycelial growth (Plate 21)

The data regarding mycelial damage caused by *Sciara* maggots in absence/presence of various bioagents as recorded at two, four, six eight and ten days of inoculation have been placed in Table 40.

An increased level of damage to the mycelium of *P. sajor caju* by *Sciara* maggots with passage of time was recorded. The mean per cent mycelial depreciation of 13.1 per cent increased significantly to 18.9, 24.7, 29.0 and 32.7 per cent at respective time duration of two, four, six, eight and ten days. All the treatments were significantly effective against the insect and caused significantly lower mean mycelial diminish with respect to control. The minimum mean mycelial depletion of 10.6 per cent was observed in T10 wherein *H. indica* @10^3 IJs were inoculated on fully impregnated mycelium grown on NSKE treated medium. Interestingly, all the treatments, though, highly effective as compared to control, produced significantly diverse mean mycelial depletion that varied from minimum of 10.6 (T10) to35.5 per cent in T3. Among solo applications of Neem products, NSKE application resulted in significantly lower mycelial depletion of 13.1, 18.5, 22.5, 27.5 and 31.6 per cent as compared to 20.7, 26.6, 31.8, 36.2, and

Control | NSKE (1%) | NSKP (1%) | *S. carpocapsae* (10^2IJs)

S. carpocapsae (10^3IJs) | *H. indica* (10^2 IJs) | *H. indica* (10^3IJs) | NSKE+ *S. carpocapsae* (10^2IJs)

NSKE+ *S. carpocapsae* (10^3IJs) | NSKE+ *H.indica* (10^2IJs) | NSKE+ *H.indica* 10^3IJs) | NSKP+ *S. carpocapsae* (10^2IJs)

NSKP+ *S. carpocapsae* (10^3IJs) | NSKP+ *H.indica* (10^2IJs) | NSKP+ *H. indica* (10^3IJs)

Plate 21: Effect of Neem product and entomopathogenic nematodes on *Sciara* sp. affecting the mycelial growth of *Pleurotus sajor caju* (*in vitro*)

41.6 per cent caused by NSKP at two, four, six eight and ten day, respectively. Similarly, among lone inoculations of entomophagous nematodes, *H. indica* produced better results than *S. carpocapsae* when compared at their respective inocula at all durations of time. Better results were obtained in the treatments receiving Neem products and entomophagous nematodes in combination as compared to those receiving either of them singly. The only exception was T6 in

Table 40 Effect of Neem product and entomopathogenic nematodes on *Sciara* sp. affecting the mycelial growth of *P. sajor caju* (*in vitro*)

Treatment	Per cent mycelial damage (days)					Mean
	2	4	6	8	10	
T1 (NSKE 1.0%)	13.1 (21.2)	18.5 (25.5)	22.5 (28.3)	27.5 (31.6)	31.6 (34.2)	**22.7 (28.2)**
T2 (NSKP 1.0%)	20.7 (27.1)	26.6 (31.0)	31.8 (34.4)	36.2 (36.9)	41.6 (40.2)	**31.4 (33.9)**
T3 (*S. carpocapsae* 1 x 10^2)	19.1 (25.9)	26.5 (30.9)	39.2 (38.7)	44.8 (41.9)	47.9 (43.8)	**35.5 (36.3)**
T4 (*S. carpocapsae* 1 x 10^3)	9.9 (18.3)	12.9 (21.1)	19.5 (26.2)	24.1 (29.4)	25.9 (30.6)	**18.5 (25.1)**
T5 (*H. indica* 1 x 10^2)	14.4 (22.3)	24.3 (24.5)	28.0 (31.9)	32.7 (24.9)	35.6 (36.6)	**27.0 (31.0)**
T6 (*H. indica* 1 x 10^3)	8.2 (16.6)	12.9 (21.1)	17.8 (24.9)	21.2 (27.4)	22.9 (28.6)	**16.7 (23.7)**
T7 (NSKE + *S. carpocapsae* 1 x 10^2)	13.6 (21.6)	17.8 (24.9)	20.9 (27.2)	25.4 (30.2)	28.9 (32.5)	**21.3 (27.3)**
T8 (NSKE + *S. carpocapsae* 1 x 10^3)	6.9 (15.3)	11.4 (19.7)	14.9 (22.7)	17.3 (24.6)	19.9 (26.5)	**14.1 (21.8)**
T9 (NSKE + *H. indica* 1 x 10^2)	10.5 (18.9)	15.7 (23.3)	19.6 (26.3)	22.8 (28.6)	25.3 (30.2)	**18.7 (25.4)**
T10 (NSKE + *H. indica* 1 x 10^3)	5.8 (13.6)	9.4 (17.7)	11.3 (19.6)	12.9 (21.0)	14.7 (22.5)	**10.6 (18.9)**
T11 (NSKP + *S. carpocapsae* 1 x 10^2)	11.2 (19.4)	16.2 (23.8)	21.1 (27.3)	23.8 (29.2)	26.1 (30.7)	**19.7 (26.1)**
T12 (NSKP + *S. carpocapsae* 1 x 10^3)	8.5 (16.9)	12.9 (21.0)	15.8 (23.5)	19.6 (26.3)	25.7 (30.4)	**16.5 (23.6)**
T13 (NSKP + *H. indica* 1 x 10^2)	10.8 (19.2)	16.4 (23.9)	20.6 (26.9)	26.3 (30.8)	30.6 (33.6)	**20.9 (26.9)**
T14 (NSKP + *H. indica* 1 x 10^3)	7.9 (16.3)	13.1 (21.2)	16.2 (23.8)	19.5 (26.2)	21.1 (27.3)	**15.6 (22.9)**
T15 (Untreated Control)	36.39 (36.4)	50.1 (45.1)	70.4 (57.1)	81.6 (64.6)	92.1 (73.7)	**66.2 (55.5)**
Mean	**13.1 (20.6)**	**18.9 (25.3)**	**24.7 (29.3)**	**29.0 (32.2)**	**32.7 (34.8)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$ Time interval (I) 0.56 Treatments (T) 0.96 I x T 2.15

which *H. indica* @10^3 IJs in lone application reduced the mycelial damage just to 16.7 per cent, signifying the high efficiency of this nematode species against *Sciara* maggots.

Table 41 Effect of Neem product and entomopathogenic nematodes on population of *Sciara* sp. *P. sajor caju* (*in vitro*)

Treatment	Per cent larval mortality (days)					Mean
	2	4	6	8	10	
T1 (NSKE 1.0%)	28.3 (32.1)	46.7 (43.1)	51.7 (45.9)	55.0 (47.9)	56.7 (48.8)	**47.7 (43.6)**
T2 (NSKP 1.0%)	15.0 (22.6)	26.7 (31.1)	30.0 (33.1)	36.7 (37.1)	41.7 (40.2)	**30.0 (32.8)**
T3 (*S. carpocapsae* 1 x 10^2)	11.7 (19.9)	21.7 (27.7)	31.7 (34.2)	43.3 (41.1)	61.7 (51.8)	**34.0 (34.9)**
T4 (*S. carpocapsae* 1 x 10^3)	28.3 (32.1)	51.7 (45.9)	55.0 (47.8)	56.7 (48.8)	58.3 (49.8)	**50.0 (44.9)**
T5 (*H. indica* 1 x 10^2)	20.0 (26.4)	33.3 (35.0)	43.3 (41.9)	55.0 (47.9)	65.0 (53.8)	**43.3 (40.9)**
T6 (*H. indica* 1 x 10^3)	50.0 (44.9)	65.0 (53.8)	71.7 (57.9)	73.3 (58.9)	78.3 (62.4)	**67.7 (55.7)**
T7 (NSKE + *S. carpocapsae* 1 x 10^2)	40.0 (39.2)	51.7 (45.9)	61.7 (51.8)	68.3 (55.8)	73.3 (59.0)	**59.0 (50.4)**
T8 (NSKE + *S. carpocapsae* 1 x 10^3)	53.3 (46.9)	60.0 (50.9)	66.7 (54.8)	71.7 (57.9)	80.3 (63.5)	**66.3 (54.4)**
T9 (NSKE + *H. indica* 1 x 10^2)	38.3 (38.2)	53.3 (46.9)	70.0 (56.8)	73.3 (58.9)	75.0 (60.1)	**66.3 (56.3)**
T10 (NSKE + *H. indica* 1 x 10^3)	53.3 (46.9)	61.7 (51.8)	68.3 (55.7)	76.7 (61.1)	83.3 (66.1)	**68.7 (56.3)**
T11 (NSKP + *S. carpocapsae* 1 x 10^2)	35.0 (36.1)	50.0 (44.9)	55.3 (47.9)	58.3 (49.8)	65.3 (53.7)	**52.7 (46.5)**
T12 (NSKP + *S. carpocapsae* 1 x 10^3)	40.0 (39.2)	55.3 (47.8)	61.7 (51.7)	66.7 (54.7)	73.3 (58.9)	**59.3 (50.5)**
T13 (NSKP + *H. indica* 1 x 10^2)	48.3 (44.1)	53.3 (46.9)	66.7 (54.8)	70.0 (56.8)	71.7 (57.9)	**62.0 (52.1)**
T14 (NSKP + *H. indica* 1 x 10^3)	50.0 (44.9)	55.0 (47.9)	61.7 (51.8)	70.0 (56.8)	73.3 (58.9)	**62.3 (52.1)**
T15 (Untreated Control)	1.7 (4.3)	3.3 (8.6)	3.3 (8.6)	5.0 (12.9)	6.7 (14.8)	**5.3 (9.8)**
Mean	**34.2 (34.6)**	**45.9 (41.9)**	**53.2 (46.3)**	**58.7 (49.8)**	**64.2 (53.3)**	

Figures in parentheses are arc sine transformed values

$CD_{(0.05)}$	Time interval (I)	1.42
	Treatments (T)	2.46
	I x T	5.51

4.8.5.2 Effect on larval mortality of *Sciara* sp.

Data placed in Table 41 is suggestive of the fact that although all the formulations tested against *Sciara* maggots showed significant efficacy as compared to control as far as effect on *Sciara* maggots are concerned. Significantly increased number of maggots faced mortality in all the treatments as compared to control at all durations of time. The larval mortality increased with time as the mean per cent kill of 34.2 at two days enhanced to 64.2 by 10 days. Highest mean insect mortality of 68.7 recorded in T10 was statistically similar to 66.3, 66.3 and 67.7 per cent achieved in T8, T9 and T6, respectively, thus indicating all these treatments to be equally effective against the test insect in oyster mushroom. Treatment receiving combination of Neem product and entomophagous nematodes (T7 to T14) produced significantly higher insect mortality than the treatments receiving solitary application of either of the two. Whereas natural larval mortality to the tune of 6.7 per cent was observe in untreated control by 10 days, highest per cent kill of 83.3 per cent was obtained in T10 applied with combination of NSKE and *H. indica* @10^3 IJs per plate at this time. This percent kill was at par with 80.3 per cent recorded in T8 having combination of NSKE and *S. carpocapsae* @10^3 IJs per plate. Fastest insect kill was also recorded in these treatments with 53.3 per cent mortality at two days. The assessment of data indicated both the nematodes to be equally effective against test insect when used in combination with NSKE. However, in solitary application *H. indica* incurred significantly more number of insect deaths as compared to *S. carpocapsae* at both inocula of 10^2 and IJs identifying former to be the better bioagent than the later.

4.8.5.3 Correlation between larval mortality and mycelial depletion of sciarid

Sciarid maggots shared a positive relationship with the mycelial depletion. More the *number of larval mortality, lesser was the mycelial depletion of P. sajor caju, suggestive of a* negative correlation between larval mortality and mycelial depreciation as evident from data assembled in Table 42.

Table 42 Correlation between larval mortality and mycelial depletion of sciarid

	Mycelial Depletion	**Larval Mortality**
Mycelial Depletion	1.000	
Larval Mortality	-0.92*	1.000

*Significant at 5 % level of confidence

In vivo

4.8.6 Effect on yield of *P. sajor caju* (Spawning time inoculation)

Statistical revelations represented in Table 43 were indicative of high susceptibility of oyster mushrooms to sciarid maggots especially when infestation occurred in initial phase of cropping. Sporocarp production declined significantly to 215.0 g in insect inoculated control as compared to 361.7 g weighed in insect free untreated bags. Sporocarp yields improved significantly in all the treatments with respect to insect inoculated control (T15) except T2, T11 and T13 with respective yields of 245.0, 250.0 and 248.3 which were statistically as low as

Table 43 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the yield of *P. sajor caju* (Spawning time inoculation)

Treatment	**Yield per bag (g)**	**Yield loss (%)**
T1 (NSKE @ 1.0%)	315.0	12.9
T2 (NSKP @ 1.0%)	245.0	32.3
T3 (*S. carpocapsae* @ 1 x 10^4)	286.7	20.7
T4 (*S. carpocapsae* @1 x 10^5)	313.3	13.4
T5 (*H. indica* @1 x 10^4)	266.7	26.3
T6 (*H. indica* @1 x 10^5)	323.3	10.6
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	291.7	19.4
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	303.3	16.1
T9 (NSKE + *H. indica* @ 1 x 10^4)	280.0	22.6
T10 (NSKE + *H. indica* @ 1 x 10^5)	318.3	12.0
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	250.0	30.9
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	265.0	26.7
T13 (NSKP + *H. indica* @ 1 x 10^4)	248.3	31.3
T14 (NSKP + *H. indica* @ 1 x 10^5)	260.0	28.1
T15 (Untreated inoculated control)	215.0	40.6
T16 (untreated uninoculated control)	361.7	-
C.D.	**39.94**	**-**

control. However, quantum of fruiting bodies produced in all other treatments was significantly lower than 361.7 produced in T16 barring T6 in which sporocarp production of 323.3 g was statistically at par with former. Sporocarp yields in T1 (315g), T4 (286.7), T5 (313.3), T7 (291.7), T8 (303.3) and T10 (318.3), though statistically not equivalent to 361.7g achieved in T16, were at par with 323.3 g produced in T6. It could be inferred from these observations that NSKE or entomophagous nematodes of both the genera in solo applications as well as in combination were significantly effective against the test insect that led to improvement of the sporocarp yields. NSKP on the other hand, in individual treatment or in combination was not as effective.

Yield losses of 40.6 per cent suffered in insect inoculated control reduced considerably to 10.6, 12.0, 12.9 and 13.4 per cent in T6, T10, T1 and T4, respectively indicative these treatments to be highly effective. Yield losses in other treatments varied from 16.1 in the treatment inoculated with 10^5 IJs of *S. carpocapsae* in NSKE treated substrate (T4) to 32.3 per cent in the treatment receiving NSKP application (T2).

4.8.7 Effect on yield of *P. sajor caju* (inoculation at pin head formation stage)

The data recorded in Table 44 when statistically scrutinized showed non significant reduction in sporocarp production when sciarids infested the crop at later stage of pin head formation. Per cent yield decline in insect inoculated control with respect to uninoculated check was 12.1 per cent. Quantitative losses remained in a range of 3.3 to 10.0 per cent in the treatments receiving Neem products and/or entomopathogenic nematodes as bio control agents. **4.8.8 Effect on yield of *P. sajor caju* (spawning and pin head formation stage inoculation)**

The observations recorded in Table 45 revealed tremendous decline in sporocarp emergence when insect maggots were inoculated in two phases viz., at spawning as well as at pin head formation stage. Barely 140.0 g of sporocarps were yielded in untreated insect inoculated bags as compared to 346.7 g emerging in insect free control bags. Though sporocarp production enhanced significantly in all the treatments with respect to insect inoculated control, it was not at par with uninoculated control in any of the treatments. Among the treatments, highest yield of 290.0 g was obtained in T4 receiving 10^5 IJs of *S. carpocapsae* which was at par with 285.0, 285.0, 283.3 278.0, 255.0, 241.7 and 241.7 g of sporocarps grown in respective treatments of T6, T8, T10, T1, T7, T3 and T9. These observations implied NSKE, *S. carpocapsae* and *H. indica* at inoculum level of 10^5 IJs alone or in combination as significantly better treatments as compared to those where NSKP alone or in combination with lower inoculum of entomophagous nematodes was used. Even lower inoculum of 10^4 IJs of any of the nematode was not sufficient to effect the test insect population significantly.

Table 44 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the yield of *P. sajor caju* (inoculation at Pin head formation time)

Treatment	Yield per bag (g)	Yield loss (%)
T1 (NSKE @ 1.0%)	383.3	4.2
T2 (NSKP @ 1.0%)	365.0	8.8
T3 (*S. carpocapsae* @ 1 x 10^4)	370.0	7.5
T4 (*S. carpocapsae* @1 x 10^5)	380.0	5.0
T5 (*H. indica* @1 x 10^4)	371.7	7.1
T6 (*H. indica* @1 x 10^5)	386.7	3.3
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	375.0	6.3
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	380.0	5.0
T9 (NSKE + *H. indica* @ 1 x 10^4)	370.0	7.5
T10 (NSKE + *H. indica* @ 1 x 10^5)	383.3	4.2
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	365.0	8.8
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	366.7	7.1
T13 (NSKP + *H. indica* @ 1 x 10^4)	360.0	10.0
T14 (NSKP + *H. indica* @ 1 x 10^5)	370.0	7.5
T15 (Untreated inoculated control)	351.7	12.1
T16 (untreated uninoculated control)	400.0	-
C.D.	**N.S.**	-

Sporocarp yield losses assessed to the level of 59.6 per cent in untreated insect inoculated control declined to the minimum of 16.4 per cent in T4 receiving 10^5 IJs of *S. carpocapsae*. These losses were considerably similar to 17.8, 17.8, 18.3 and 19.7 per cent in T6, T8, T10 and T1, respectively. Yield losses in all other treatments were above 26.0 per cent (Table 45).

Table 45 Effect of Neem products and entomophagous nematodes on *Sciara* population affecting the yield of *P. sajor caju* (dual inoculation at spawning + pin head formation time)

Treatment	Yield per bag (g)	Yield loss (%)
T1 (NSKE @ 1.0%)	278.3	19.7
T2 (NSKP @ 1.0%)	193.3	44.2
T3 (*S. carpocapsae* @ 1 x 10^4)	241.7	30.3
T4 (*S. carpocapsae* @1 x 10^5)	290.0	16.4
T5 (*H. indica* @1 x 10^4)	231.7	33.2
T6 (*H. indica* @1 x 10^5)	285.0	17.8
T7 (NSKE + *S. carpocapsae* @ 1 x 10^4)	255.0	26.4
T8 (NSKE + *S. carpocapsae* @ 1 x 10^5)	285.0	17.8
T9 (NSKE + *H. indica* @ 1 x 10^4)	241.7	30.3
T10 (NSKE + *H. indica* @ 1 x 10^5)	283.3	18.3
T11 (NSKP + *S. carpocapsae* @ 1 x 10^4)	218.3	37.0
T12 (NSKP + *S. carpocapsae* @ 1 x 10^5)	240.0	30.8
T13 (NSKP + *H. indica* @ 1 x 10^4)	220.0	36.5
T14 (NSKP + *H. indica* @ 1 x 10^5)	243.3	29.8
T15 (Untreated inoculated control)	140.0	59.6
T16 (untreated uninoculated control)	346.7	-
C.D.	**44.21**	-

CHAPTER-5

DISCUSSION

Cultivated mushrooms are the luscious fleshy fungi in which elegant tender edible sporophores develop out of the fragile mycelium. Being extremely fine and delicate in texture, both the mycelium and fruiting bodies are highly vulnerable to the offence of various pests and pathogens. Another cause of this inordinate susceptibility is the conditions prevailing in the closed biological system of mushroom cultivation that are highly favorable for these pests. Unhygienic conditions prevailing in and around the mushroom units add to their menace. Insects, nematodes, mites and springtails constitute the important groups of mushroom pests. Of these, insects belonging to orders viz., Diptera and Coleoptera are of paramount significance. Order Diptera is represented by the flies belonging to six families viz., Sciaridae, Phoridae, Cecidomyiidae, Scatopsidae, Drosophilidae and Sphaeroceridae (Thomas, 1942; Weigel, 1959; Hussey, 1969; White, 1982; Yang and Zhang, 1987; Lewandowski *et* al., 1999; Navarro *et al*., 2000, Smith and Gupta, 2002; Deepthi *et al*., 2003; Greenslade and Clift, 2004 and Jesse, 2005). A few genera of beetles belonging to different families of Coleoptera frequently prevail in the cropping beds (Ashe, 1990; Johal *et al*., 1992; Lewandowski *et* al., 1999; Deepthi *et* al., 2004; Kumar, 2006; Mazumdar *et al*., 2008). These pests incur pronounced qualitative as well as quantitative losses to mushrooms all over the world (Zaayen, 1978; Sandhu and Brar, 1980; Kielbasa and Snetsinger, 1981; White, 1986; Aggarwal, 2000; Clift and Terras, 2000; Kumar and Sharma, 2000). The investigations carried out till date are scattered in nature and are confined mainly to *Agaricus bisporus*. Present investigations were thus undertaken to study the insect pest and spring tail fauna of two commercially grown mushrooms of Himachal Pradesh viz., white button mushroom, *Agaricus bisporus* (Lange) Imbach and oyster mushroom, *Pleurotus sajor caju* Singer.

A faunistic survey was conducted to acquaint with the insect pest status in two referred mushrooms. Twenty three button mushroom growing units and nine oyster mushroom cultivating farms located in nine mushroom growing districts of Himachal Pradesh were covered under the survey studies. Sample analysis revealed the prevalence of dipteran and coleopteran pests in most of the mushroom farms barring one located at Paonta Sahib as this was a purposely built, commercial mushroom farm fulfilling all the requirements of hygiene that prohibited the ingress of insect pests. Since, all other farms located at Darin, Samlohal, Kandror (Distt. Bilaspur), Mansai, Haretha, Kuthera, Kango (Distt. Hamirpur), University mushroom farm, Sehal, Gander (Distt. Kangra), Kathiyala, Rampur 17 Mile, Larenkelo (Distt. Kullu), Dhangiyara, Sohar, Sandhol (Distt. Mandi), Fagu, Theog, Shimla (Distt. Shimla), Maryog, Dumki (Distt. Sirmaur), Nauni, Shamlech, Jatoli, Oachghat (Distt. Solan) and Una (Distt. Una) were makeshift houses managed by marginal farmers to grow mushrooms on seasonal basis, they harbored large number of insect pests because of absence of basic growing amenities and unhygienic conditions prevailing in and around the units. Among these, insects belonging to two orders i.e. Diptera and Coleoptera were predominant. Order Diptera was represented by the insects belonging to genera *Sciara* sp. (Family Sciaridae) and *Megaselia* sp. (Family Phoridae). *Sciara* sp. though earlier reported from outside the country has been reported for the first time from the state. In addition to these genera, occurrence of insects representing some other families like Scaptosidae, Sphaeroceridae, Cecidomyiidae and Drosophilidae have earlier been reported from elsewhere in the globe (White, 1982; Lewandowski *et al*., 1999; White and Smith, 2000; Smith and Gupta, 2002; Greenslade and Clift, 2004; Deepthi *et al*., 2004; Jesse, 2005; Kumar, 2006).

Four genera of beetles belonging to four families viz., *Cyllodes indicus* (Family Nitidulidae), *Spondotriplax pallidipes* (Family Erotylidae), *Scaphisoma nigrofasciatum* (Family Scaphididae) and *Staphylinus* sp. (Family Staphilinidae) were encountered in the cropping bags and on the fruiting bodies of the mushrooms. Of these coleopteran pests, *S. pallidipes* was found damaging sporocarps of oyster mushroom. This is the first ever record from mushrooms in the world. Other beetles have earlier been reported from mushrooms from varied locations (Ashe, 1990; Asari *et al*., 1991; Johal *et al*., 1992; Lewandowski *et al*., 1999; Deepthi *et al*., 2004; Kumar, 2006, Mazumdar *et al*., 2008). The incidence of *C. indicus* has been recorded for the first time, damaging both button and oyster mushrooms, in Himachal Pradesh. In addition, the spring tails belonging to two genera viz., *Achorutes armatus* and *Lepidocyrtus cyaneus* prevailed in the dark and damp areas of the cropping rooms in most of the locations. Of these, *A. armatus* occurred more commonly and in higher numbers as compared to *L.* cyaneus. Both of these genera of springtails have been noticed in the farms of the state earlier to present revelations also (Kumar and Sharma, 1997; Kumar, 2006). These observations substantiate the evidence of wide distribution of insect pests in mushrooms in the state wherever the conditions are far from hygienic.

Among the insect pests, most significant were sciarid flies that registered their presence in 71 out of total of 87 samples of *A. bisporus* collected from 22 mushroom farms. Maggots of these flies infested compost, top dressing (= casing), mycelium as well as sporophores. Adults were found hovering over the bags but did not cause damage. Maggot population of sciarids varied from 3-85/250 cc sample; lowest being in Kathiyala (Distt. Kullu) and highest in Sehal (Distt. Kangra). Adult population wherever recorded was above the threshold level of one, thus indicating their presence above economic injury level in these farms. Phorids of *Megaselia* sp. were not found to be as common as sciarids. Their prevalence was recorded in three units only out of the total of 23 surveyed. The maggot population varied from 5-29/250 cc sample. Lowest count of five maggots was recorded in Nauni (Distt. Solan) and highest count of 29 was observed in University farm, Kangra. These observations are in variance to some earlier records as per which both sciarids and phorids occur predominantly in mushroom farms (Kumar and Sharma, 2001a; Kumar *et al*., 2008). The unprecedented low prevalence of phorids during present investigations could be attributed to the season of the survey that was conducted mainly during winter season when marginal growers of the state grow one or two crops mostly under near natural conditions. The prevailing temperature conditions during that period are more suitable for sciarids than phorids. Sciarids and phorids have near ubiquitous distribution even in distantly located mushroom growing nations (Weigel, 1959; Sasakawa and Akamatsu, 1978; Sandhu and Brar, 1980, Clift, 1981; Bhandari and Singh, 1986; Chakaravarty *et al*., 1987; Mohan and Disney, 1995; Alekseeva and Sukolova, 1996; Navarro *et al*., 2000; Greenslade and Clift, 2004; Kumar, 2006).

White button mushrooms harbored three genera of beetles viz., *C. indicus*, *Staphylinus* sp. and *S. nigrofasciatum*. The most frequently occurring was *Staphylinus* sp. recorded from 14 of the 23 locations surveyed. Highest population of 16 grubs and nine adults of this beetle were encountered in Samlohal (Distt. Bilaspur). Though, *C. indicus* was found to prevail only in nine distantly located mushroom units, its population was generally higher. Peak population of this beetle was observed in Sohar (Distt. Mandi). Twenty three grubs and 18 adults of this beetle were trapped from 250 cc compost sample collected from a cropping bag. Another beetle *S. nigrofasciatum* was of the rare occurrence and their grubs as well as adults were seen in small numbers in five locations out of 23 locations surveyed. It is fascinating to put on record that no other beetle barring *S. nigrofasciatum* has been reported from *A. bisporus* prior to present investigation. Deepthi *et al*. (2004) reported the presence of this beetle in white button mushroom. All these beetles and their grubs were found to feed voraciously upon the sporophores to leave them mushy to the extent that they no more remained marketable. Adults also fed upon the mycelium and devitalized it completely. It is surprising as to why these beetles have not attracted the attention of active mushroom protection researchers so far.

Cultivation of oyster mushroom was not as frequently undertaken by the growers of the state. As such nine farms located in six districts growing this mushroom were surveyed. Analysis of 18 substrate samples collected from the cropping bags revealed the ubiquitous distribution of insect pests in *P. sajor caju* as all the samples showed the infestation of sciarids. Sizeable counts of pre-pupae and pupae were also observed. Adults were found hovering over the bags in most of the locations. Looking into their abundance, traps were laid at selected farms located nearby at Nauni (Distt. Solan) and Maryog (Distt. Sirmaur). Thousands of adults were captured on these traps during the growing season. However, phorids (*Megaselia* sp.) registered their presence only in Nauni and Palampur (Kangra), University mushroom farms.

Beetles were also more abundant in oyster mushrooms. Coleopteran fauna in *P. sajor caju* was same as found in *A. bisporus*. The most widely distributed was *S. nigrofasciatum* as its presence was revealed in eight out of nine locations. Shamlech mushroom farm was free of most of the insect pests including this beetle as it worked commercially under near ideal conditions. Interestingly, this was the beetle of rare occurrence in *A. bisporus*. *Cyllodes indicus* and *Staphylinus* sp. also occurred in seven out of nine places. Presence of these beetles in oyster mushroom has also been reported prior to this from different parts of the country (Asari *et* al., 1991; Johal *et al*., 1992; Mazumder *et al*., 2001; Deepthi *et al*., 2004; Kumar; 2006). All these beetles were menacing for the crop as their grubs fed upon the sporocarps and destroyed them and their adults were voracious feeders of mycelium as well as fruiting bodies. In addition, one more beetle *Spondotriplax pallidipes* (Family Nitidulidae) was found damaging oyster mushroom in two nearby locations of Oachghat and Nauni (Distt. Solan). Recovery of 48 grubs and 12 adults/250 cc substrate sample from the bag yielding almost no sporocarp production indicated that these beetles may turn to be serious pests in near future. Prevalence and suspected damaging potential of these beetles signified the need of future research on these insects.

Among insect pests of white button mushroom, the most abundant were sciarid flies with 33.0 to 100.0 per cent frequency of incidence. *Megaselia* sp., was not as widely distributed but the two farms of Kangra and Nauni where they showed their presence, 83.3 and 80.0 per cent samples, respectively, were found to be infested. Beetles were less abundant than flies. *Staphylinus* sp. was the most commonly occurring beetle infesting 50.0 per cent samples with frequency of incidence varying from 20.0 to 75.0 per cent in various locations. *Cyllodes indicus* frequented 38.9 per cent samples. The minimum of 25.0 per cent samples collected from Una and Samlohal and maximum of 75.0 per cent samples collected from Sohar showed its infestation. *Scaphisoma nigrofasciatum* was of least abundance in *A. bisporus* with 27.8 per cent frequency of distribution.

The insect fauna of *P. sajor caju* was similar to that of *A. bisporus* but for an additional beetle *S. pallidipes* that frequented the former but not later. Comparative observations corroborate that oyster mushrooms were more susceptible to insect pests than white button mushrooms especially with respect to beetles. The probable reason for this inordinate susceptibility could be the open gilled structure of oyster mushroom sporocarp that facilitated the easy movement of grubs and adults and provided sufficient hiding space in the gills. On the other hand, smooth outer surface of pileus of *A. bisporus* was not as fascinating.

Two genera of springtails were encountered in the mushroom farms wherever dark and humid conditions prevailed in the cropping rooms. *Achorutes armatus* was more widely distributed as it infested 58.1 per cent samples as compared to *L. cyaneus* which was present in 25.8 per cent samples only. The overlapping populations of both the genera were retrieved from locations like Darin and Larenkelo. Oyster mushroom was more vulnerable to the assault of these tiny collembolans as compared to button mushrooms. Infestation to the extent of 87.5 per cent was observed in former as compared to 65.2 per cent in later. Probably the higher temperature conditions during cultivation of oyster mushrooms were more conducive for these tiny organisms. Bhandari and Singh (1983); Kumar and Sharma (1997) and Kumar, 2006 also reported these organisms from these mushrooms.

Mushroom cultivation is an enclosed biological system wherein organic wastes are decomposed under humid conditions that invite number of pests. These pests thrive and multiply manifold in this system as mushroom growing conditions are just ideal for them. This is the reason for preponderance of insect, collembola and mite fauna in cultivated mushrooms.

The predominant insect and collembola fauna collected from different locations were brought to laboratory and were identified with the help of available literature and taxonomic keys. The insects retrieved were identified as *Sciara* sp., *Megaselia* sp., *C. indicus*, *Staphylinus* sp., *S. nigrofasciatum* and *S. pallidipes*. The collembola fauna comprised of *A. armatus* and *L. cyaneus*. The test insect was identified as *Sciara* sp. on the basis of taxonomic characters and the identification was got confirmed from the taxonomist Dr. V. V. Rama Murthy, Division of Entomology at IARI.

Sciara eggs collected from *A. bisporus* mycelium were microscopic, smooth and oval in shape averagely measuring 0.21 mm in length and 0.15 mm in breadth. Eggs were dirty white initially and fertile eggs turned transparent by the end of incubation period. Infertile eggs turned dark and shriveled. The mean egg viability was 89.9 ± 4.8 per cent. The newly hatched maggots were cylindrical with a shining black head and body tapering towards the posterior end. On an average they measured 0.57 mm in length and 0.08 mm in breadth. The maggots initially fed upon the thickened mycelial attachments, made tunnels while voraciously feeding on mycelial sap and detached the attachments, thus, impairing further mycelial impregnation. The maggots matured through four larval instars within 13-14 days to reach the average length of 5.58 mm and breadth of 0.50 mm. The differentiation of instars was made on the basis of head capsules retrieved at 2-3, 5-6, 8-9 and 12-14 days. The matured maggots turned into non-feeding pre-pupae that looked shriveled with appendages articulated to their body. Pre-pupae were smaller than matured maggots with average size of 2.51 x 0.26 mm. Pre-pupal phase lasted for 1-3 days. Newly formed pupae were dirty white in color which changed to yellowish brown after 1-2 days. The matured pupae were deep grey in color with distinctly visible compound eyes and appendages and measured 1.92 - 2.70 mm in length and 0.50-0.70 mm in breadth. The average per cent survival rate of pupae was 82.0 ± 8.9 per cent. The newly emerged flies were grayish black with elongated abdomen, long legs with contiguous coxae and thread like filiform antennae having 14 annuli in the flagellum. Wing venation comprised of costa and sub costa which fused to form thick coastal margins in for wings. Thick radial and radial sector veins were joined by a radial cross vein. The stem of median vein was faint and this vein forked into M_{1+2} and M_3. Cubitus vein forked into Cu_1 and Cu_2. Abdomen of male fly was slender and shorter than female and terminated into claspers. Female abdomen was swollen and terminated into a pointed ovipositor.

The adult females had better longevity than males. An average female survived for 6.0 ± 0.7 days as compared to an average male's longevity of 5.17 ± 0.8 days. Copulation under captivity was observed when they mated on the mycelium in the Petri plates. They were observed end to end 'in copula' position that broke once or twice before actual mating. During this period, female remained stable but male made jerky movements around her. Actual mating took place thereafter. Mating period varied for 4-8 minutes. The male moved swiftly after mating but female remained inactive. Female started ovipositing after 15-26 hours of mating. Eggs were laid singly or in clusters of 8-12. Average fecundity was 29-58 eggs/female. Post oviposition was very small as female survived barely for few hours after oviposition. The developmental phase from egg to adult emergence was completed in 13.67 days at 23 ± 1^{o}C on *A. bisporus.*

Biological parameters of *Sciara* sp. when developed on *P. sajor caju* at 26 ± 1^{o}C exhibited a delay of three days in completion of insect development from egg to adult. The development from egg to adult on oyster mushroom completed averagely in 16.7 days. Development of the insect must have been delayed in oyster mushroom due to higher temperature as other growing conditions including humidity were similar for both the mushrooms. The observations signified the influence of temperature on development of the test insect.

In natural populations number of females was always more than that of males. Respective sex ratios of 1.41 and 1.29 were assessed in the populations feeding upon *A. bisporus* and *P. sajor caju.*

Megaselia sp. commonly known as humpbacked fly/schuttle fly was identified as its adults had humpback, rudimentary antennae sunken in the head cavity. Coxae in the legs were contiguous with each other and hind femora were robust and laterally flattened. Their freshly hatched maggots were transparent with visible thread like dorsal tracheal vessel of whitish hue.

Adults of *Staphylinus* sp. (Rove beetles) were slender, elongated, black beetles having enlarged moniliform antennae arising on front of the head nearer to eyes and had characteristic short elytra which were rounded at the apex. These beetles ran swiftly, frequently raising their abdomen just like scorpions while running. Six to eight sternal segments were exposed beyond elytra.

Adults of *Scaphisoma nigrofasciatum* were shiny beetles, pointed at both the ends and had filiform antennae. The elytra was short and truncate at apex and only one or two abdominal sterna remained exposed beyond elytra.

Cyllodes indicus were small, oval beetles with club shaped three segmented antennae and shiny black elytra cut off squarely at the apex exposing one or two apical abdominal segments. Elytra had two orange spots.

Spondotriplax pallidipes often referred as pleasing fungus beetles were medium sized oval shining beetles with three to four segmented clubbed antennae and smooth elytra. This beetle has been reported from oyster mushroom for the first time.

Out of two genera of collembolans harboring cropping rooms of mushroom, *Achorutes armatus* were small, soft bodied, silver grey spring tails with rounded head having two black ocellar fields bearing rudimentary ocelli and minute hairs covering the entire body. These spring tails were distinguished due to the absence of 'furcula'. *Lepidocyrtus cyaneus* on the other hand were minute, brown colored spring tails with rudimentary ocelli and a collophore, a tenaculum and a furcula each on first, third and fourth abdominal segment, respectively.

Studies on nature of damage of sciarid and phorid flies revealed that their maggots voraciously fed upon the mycelium and caused severe depletion. The flies damaged severely the developing primordia or pin heads wherein mycelial attachments were severed by the maggots resulting into browning of newly emerging pin heads which became leathery and never developed into sporocarps. The infested pin heads/sporocarps became hollow and spongy. The maggots also entered the stipe of matured sporophores from its base and caused tunneling as they moved from the stipe to pileus.

Phoretic behavior was observed frequently in nematodes, mites and some small unidentified arthropods. Nematodes and mites congregated on the surface of cropping medium in large numbers and clinged to the legs, thorax and abdomen of adult insects associated with mushrooms that included flies and beetles. These insects acted as carriers to disseminate these harmful pests to the healthy cropping beds/rooms. Data collected on phoretic behavior of mites and nematodes during present investigations showed lower level of phoresy by nematodes as compared to mites. This could be attributed either to the lower level of nematode contamination in the experimental beds or to the lower tendency of phoresy in nematodes in comparison to mites. Among coleopterans, the highest level of phoresy was observed in *C. indicus*, 60 per cent tested specimens of which had nematodes and mites clinged to their bodies. This was followed by *S. nigrofasciatum* and *S. pallidipes*, each, showing 40 per cent phoresy. Also, phoresy in coleopterans was much higher than dipterans as 36.0-60.0 per cent of tested beetles were found to carry nematodes and mites on their bodies. Compared to that, among flies, only 17.1 per cent of the tested *Sciara* adults acted as carriers of phoretic organisms. Beetles acted as efficient carriers probably due to their larger size, clinging legs and their tendency to remain on the surface of the cropping medium for most of the time that provided ample opportunity to mites and nematodes to cling to their bodies. In contrast, adult flies were smaller in size and had a tendency to hover over the cropping beds rather than sitting on the bed surface for long durations. Primitive studies on phoretic behavior have earlier been conducted by workers like Norton and Ide (1974), Binns (1980a) and Chandran (2000) who reported phoresy by mites and nematodes on dipteran insects. However, only one report of phoresy on coleopteran beetles associated with mushrooms is available till date in which 33.0-100.0 per cent tested coleopterans were found to carry the nematodes and mites on their bodies as compared to 10.0-60.0 per cent dipterans acting as carriers (Kumar, 2006). The inference of present studies matches the findings of this earlier report as far as comparative phoretic intensity in beetles and flies is concerned but per cent phoresy during present investigation was appreciably lower in both the groups when compared with this report. The variations could be attributed to the factors like difference in growing conditions, temperature and humidity levels at the time of studies and level of infestation of both phoretic and carrier organisms during investigations.

Seasonal abundance of the test fly *Sciara* sp. was worked out for two years and five months w.e.f. July, 2007 to 2009. The insect remained in the farm through out the year in varying numbers. Nil to negligible population of the insect was seen in the mushroom unit under surveillance during the month of August through out the observation period. Peak population of the insect was recorded from March to May every year when average temperature ranged from 20-24 ^{o}C and relative humidity from

54.5 to 64.8 per cent. Thus, the referred temperature and relative humidity conditions were considered to be most conducive for the multiplication of *Sciara* sp. These observations are in line with the work of Kumar and Sharma (1999) who reported year round distribution of Sciarids in mushroom units but contrary to their report that highest population of sciarids occurred from February to April when temperature ranged from 11-20 ^{o}C. Kumar *et al.* (2008) also recorded that as the temperature rose from 13.8 to 21.3 ^{o}C, the number of sciarid flies decreased. These variations in the temperature requirement could be due to difference in the genus of sciarid insects as these workers worked with *Bradysia tritici* and not *Sciara* sp as in present studies. However, Kumar and Sharma (2001) recorded peak sciarid population in the mushroom farm during the months of March, April and May under Solan conditions.

Studies conducted on relative susceptibility of two mushrooms to *Sciara* sp. under laboratory conditions suggested that per cent mycelial depletion increased corresponding to increase in inoculum level as well as duration of time. Initial inoculum of two maggots caused depletion to the level of 17.8 per cent at 10 days of inoculation as compared to 63.1 per cent impairment caused at this time when 16 maggots were initially inoculated on fully impregnated mycelium of *A. bisporus*. Extent of mycelial inhibition in *P. sajor caju* at inocula of two and 16 maggots was 22.7 and 64.4 per cent, respectively, at 10 days of inoculation. Thus, damage caused to the mycelium in two mushrooms by the respective inocula was almost similar. Though, studies on effect on mycelium of mushrooms have earlier been carried out in relation to myceliophagous nematodes (Kumar *et al.*, 2007, 2007a), no experiment of this type has been conducted on entomological aspects till recent past.

These preliminary observations under laboratory conditions led to further experimentation on damaging potential of the test insect against button and oyster mushrooms in university mushroom farm under near controlled conditions.

In button mushroom, inoculum level of 2, 20 and 200 maggots/ 10kg substrate was applied separately at spawning time, casing time and at spawning + casing time. Data regarding mycelial depletion collected in *A. bisporus* at casing time, at 15, 30 and 45 days of casing revealed significant increase in mycelial depletion with the progress of time at all levels of inoculum as compared to uninoculated control. The lowest inoculum of two maggots at spawning did not inhibit mycelial growth when observed at casing time. Inocula of 20 and 200 maggots applied at this stage, however, caused significant depletion as compared to control by this time. Mycelial impairment then onwards was faster at all inocula and reached beyond 90.0 per cent in all the treatments at the time of termination except in uninoculated control wherein still 20.0 per cent of mycelium was left in the bags. Mycelial depletion after casing in insect inoculated treatments was attributed to two factors i.e. due to increase in number of insects as manifold multiplication occurred and new progeny produced, once the life cycle was complete in all the treatments as well as due to sporophore emergence as compared to uninoculated control wherein mycelium depleted only due to button production. Comparative interpretation of data revealed significantly increased mycelial depletion in all the treatments as compared to uninoculated control at all levels of inocula, thus signifying the role of insect as mycelial feeder/inhibitor. The very fact that mycelial depletion increased corresponding to increase in inoculum level at all inoculation times confirmed the role of test insect. Earlier damage potential of insects on *A. bisporus* has been evaluated only with respect to their effect on sporophore production. Observations on their effect on mushroom mycelium which eventually leads to yield losses have not been emphasized.

The maggots voraciously fed upon the mycelium, pinheads and emerging sporophores and converted to non feeding prepupae, pupae and finally adults that hovered over the bags and after mating laid eggs on the surface of cropping bags. The fact was evident as all developmental stages were found in varying numbers in the compost/ casing samples analyzed. The maggots fed upon the mycelium and severed the mycelial attachments in the process which resulted into the cessation of hyphal multiplication thus resulting in to its depletion. The infested pin heads turned brown and dried prematurely without developing in to the fruiting bodies. The maggots entered the sporophores from the base of the stipe. These sporophores when dissected showed the maggots tunneling through the stipe to the pileus on which they fed voraciously on gills thus rendering them unfit for consumption. Earlier, Jesse (2005) observed the cuts in the mycelium, reduced primordial formation and cavities in the stipe and caps of the mushroom as symptoms of Dipteran infestation in white button mushroom. Insect multiplication was density dependent and the lowest multiplication rate was observed at the highest level of initial inoculum and vice versa.

Extensive damage to the crop occurred because of *Sciara* infestation. The factors like initial inoculum of the maggots and cropping stage at which the infestation occurred significantly influenced the total sporophore production. Yield losses to the tune of 13.3, 31.5 and 53.1 per cent as compared to control were recorded at respective inocula of 2, 20 and 200 maggots when inoculated in initial phase of cropping i.e. at spawning stage. Corresponding declines in button production at casing time inoculation were 7.1, 24.2 and 49.9 per cent. Maximum decline in button production was recorded when dual maggot inoculation at spawning as well as casing time was done. Button production reduced to 1388.3, 976.3 and 740.0 at respective inocula of 2, 20 and 200 maggots as compared to uninoculated control producing 1706.7 g fruiting bodies. These yield losses, when worked out, were 18.7, 42.8 and 56.7 per cent at inoculum levels of 2, 20 and 200 maggots, respectively. Though, no research has earlier been conducted to study the damage potential of *Sciara* sp., the results stand close to the inferences met by Sandhu and Brar (1980) who assessed 66.7 per cent losses due to sciarids in mushrooms under Punjab conditions in India. Zaayen (1978) also reported yield losses to the tune of 50 per cent due to Sciarids in Netherland. Similarly the present studies confirmed the studies of Kumar *et al.* (2001) who reported higher yield losses when the pest infested the crop at spawning as compared to when infestation occurred at/after casing. Normal flush pattern of the crop was maintained at the inoculum levels of two and 20 maggots irrespective of the time of their inoculation. However, flush pattern was disturbed at the highest level of inoculum i.e. 200 maggots, especially when maggots were inoculated at spawning or at spawning as well as casing. The quantum of sporophore emergence declined progressively in these treatments from first to sixth week when the experiment was terminated. Contrary to this, buttons appeared in low and high quantities in succession of flushes at least up to fourth week in uninoculated control and treatments receiving lower inocula of insect. Disruption in flush pattern has earlier been reported due to nematodes associated with mushrooms (Khanna and Chandran, 2002a; Kumar *et al.,* 2008) but has never been exploited in case of insects associated with mushrooms and carry significance in the light of the fact that insects are the equally frequent occurring pests of cultivated mushrooms. Correlation studies assessed a highly positive relationship between larval count and mycelial impairment and a negative correlation between insect count and sporophore yields.

Oyster mushroom cropping bags were inoculated with the same inocula as button mushrooms but inoculations were done separately at spawning, pin head formation stage and at spawning as well as pin head formation stage. Since mycelial impregnation/ depletion is not very spectacular in oyster substrate bags, data regarding weekly and total sporocarp production only were recorded to assess the damage potential of *Sciara* sp. Interpretation of results inferred that oyster mushroom was highly susceptible to *Sciara* sp. that caused extensive yield losses due to its voracious feeding. Inoculum level and cropping stage at time of infestation were crucial as far as extent of damage was concerned. Even a low initial inoculum of just two maggots at spawning reduced the sporocarp production by 15.0 per cent. Minimum losses of three per cent were recorded when infestation of two maggots occurred at pin head formation. Production was not significantly adversely affected at this stage as fruiting bodies started emerging within a week of pin head formation and this period was not sufficient for insect multiplication. Maximum reduction in fruit body production was recorded with dual inoculation at spawning as well as pin head formation. Yield losses to the tune of 24.4, 46.1 and 63.1 were recorded at respective inocula of two, 20 and 200 maggots in this treatment. Losses due to *Sciara sp.* were higher in oyster mushroom than white button mushroom. The open gilled sporocarp with very small stalk probably provided ample opportunity to the maggots to move, feed and hide in the gills that made this mushroom highly susceptible. Flush pattern was disrupted at inoculum levels of 20 or more maggots. A negative correlation between the maggot count and sporocarp yield in oyster mushroom irrespective of the cropping stage at inoculation was assessed. It is interesting to note that despite higher susceptibility of *P. sajor caju* to various insect pests as compared to *A. bisporus*, the former did not draw as much attention of the researchers as the later. It could be attributed to the fact that button mushrooms were more widely grown and accepted edible mushrooms thereby attracting more research on their protection. Oyster mushrooms on the other hand despite their high nutritional value were not as widely cultivated.

Maggot infested oyster pin heads were brown and water soaked. Such pin heads rotted prematurely. Affected sporocarps were soggy and maggots were frequently spotted feeding on the gills where they formed extensive tunnels as they moved from one part to the other in the process of feeding. The infested sporocarps had a drooping tendency. The per cent of such damaged fruiting bodies increased with increase in insect inoculum level. About 12.0 per cent sporocarps were physically distorted even at the lowest inoculum of two maggots at spawning. Stage of crop at the time of infestation also had an impact on the crop health. Infestation at pin head stage resulted into 8.8 per cent unhealthy sporocarps. Assessment of per cent healthy crop at various levels and stages of sciarid infestation has been made for the first time during present investigations.

Overall, economically viable cropping period reduced by a few days in both the mushrooms due to insect infestation.

Extensive distribution and high damaging capacities of the test insect necessitated its management. Looking into the unique sensitivity of the crop, the management tactics needed to be crop as well as environment friendly. Thus, Neem products in the form of Neem Seed Kernel Extract (NSKE) and Neem Seed Kernel Powder (NSKP) and two entomopathogenic nematodes viz., *Steinernema carpocapsae* and *Heterorhabditis indica* were tried against the infective maggots of *Sciara* sp.

In vitro experiments in *A. bisporus* revealed the efficacy of Neem as well as entomopathogenic nematodes when used alone or in combinations against the test insect. Mycelial depreciation was significantly low in all the treatments as compared to control at all time intervals of observation. However, minimum mycelial impairment was recorded in the treatment inoculated with 10^3 IJs of *H. indica* in NSKE treated medium wherein only14.8 per cent mycelial depleted by 10^{th} day as compared to 89.9 per cent depletion in control occurring due to presence of *Sciara* maggots. Larval mortality was also significantly high in all the treatments as compared to control where only 3.7 per cent mortality occurred due to natural causes. A highly significant negative correlation between the maggot kill and mycelial depletion showed the effectiveness of the test biocontrol agents. Since, most of the bioagents normally found extremely effective under *in vitro* conditions fail to produce desirable results under *in vivo/*field conditions, requiring the urgency to check the efficacy of referred treatments under *in vivo* conditions was felt. Variable results of use of entomopathogenic nematodes against different insect pests of mushrooms have earlier been reported.

Under *in vivo* experiment, having spawning time inoculation of the insect larvae the treatments' receiving NSKP were not as effective in lone application as well as in combination as far as mycelial growth was concerned. These results led to the speculation that though NSKP was not myceliotoxic when incorporated directly in compost, it probably had some adverse effect on entomopathogenic nematodes which became ineffective against the maggots in its presence. However, significantly higher sporophore yields in this experiment were obtained only in the treatments receiving inocula of *H. indica* alone or in combination with NSKE. Since, the button production in the treatments receiving lone application of the nematode was at par with that of combination of NSKE and nematode, use of nematode alone was worth recommendation for effective insect control.

Steinernema carpocapsae did not prove to be as effective as *H. indica* in managing *Sciara* population in *A. bisporus*. The reason could be the higher temperature requirement for *S. carpocapsae* multiplication that could not be met during *A. bisporus* cultivation (Kim *et al*., 2001; Devi, 2008). Insect when inoculated at late stage of cropping i.e. at casing did not incur significant yield losses. Losses in sporophore yields in various treatments ranged from 3.9 to 12.9 per cent which were at par with 19.3 per cent reduction in insect inoculated untreated control. Dual inoculation of insect at spawning as well as casing led to appreciably poor mean mycelial growth in insect inoculated untreated control. This mean mycelial growth was statistically similar to that attained in NSKP treated bags leading to the conclusion that NSKP was ineffective against the insect and thus could not improve the mycelial growth of *A. bisporus*. Button production increased significantly in all the treatments as compared to untreated inoculated control wherein only 96.7 g of sporophores were produced. Yields in all the treatments were above 126.0 g. Yield losses of 13.0 per cent obtained in the treatment receiving 10^5 IJs of *H. indica* were statically similar to 18.4 and 20.1 per cent losses attained in the bags treated with 10^4 IJs of *H. indica* and NSKE alone, respectively. These results led to the conclusion that *H. indica* or NSKE at the referred doses were most suitable for management of *Sciara* sp. in case of heavy infestation.

In vitro experiments on management of *Sciara* sp. in oyster mushrooms revealed significantly reduced mycelial depreciation in all the treatments as compared to untreated control at all intervals of observation. Mycelial depletion increased significantly with progress of time in all the treatments including control but it was significantly low in the treated bags. Maximum mean depletion of 35.5 per cent mycelium recorded in the least effective treatment receiving 10^2 IJs of *S. carpocapsae* was also statistically better than untreated control in which 66.2 per cent mean mycelial depletion was observed. Also, a significantly more larval mortality was caused in all the treatments as compared to control at all durations of observations. Only 5.3 per cent larval mortality due to natural causes was recorded in control as compared to minimum of 34.0 per cent achieved in T3 (10^2 IJs of *S. carpocapsae*). Highest mean larval mortality of 68.7 per cent in T10 (10^3 IJs of *H. indica* + NSKE) was statistically at par with T6, T8 and T9, thus, signifying the efficacy of these treatments. This information reflected the significance of all the used biocontrol agents as far as their effect on maggot population was concerned.

All the treatments of *in vitro* experiment were repeated in *P. sajor caju* cropping bags to assess their *in vivo* efficacy against *Sciara* sp. indicated in form of improved sporocarp yields when insect was inoculated at spawning, at pin head formation and both at spawning and pin head formation. Bags infested with *Sciara* maggots at spawning produced significantly poor sporocarp yields in untreated inoculated control (T15) as compared to insect free untreated bags (T16). Sporocarp production improved significantly in all the treatments barring T2, T11 and T13 wherein NSKP was used alone, in combination with 10^4 IJs of *S. carpocapsae* or with 10^4 IJs of *H. indica*, respectively. All the other treatments involving NSKE alone, *H. indica* alone or in combination to each other yielded significantly better results as far as fruiting body production was concerned. Insect when inoculated at a later stage of cropping i.e. pin head formation did not affect the sporocarp production adversely as far as statistics was concerned, thus, indicating no requirement of using management tactics. Sporocarp production was reduced significantly in the bags receiving dual infestation at spawning as well as pin head formation. Heavy yield losses to the tune of 59.6 per cent were recorded in untreated inoculated control (T15). Sporocarp yields improved significantly in all the bags receiving one or the other bioagent barring those treated with NSKP alone (T2). While Richardson (1987) and Geetanjali (2008) found *Heterorhabditis* spp. more suitable than *Steinernema* spp. against mushroom fly larvae, others like Scheepmaker *et al.* (1998a ,b), Taylor(1992) and Walia *et al.* (2004)found various isolates of *Steinernema* to be highly effective. It could be interpreted from these changeable results that it was not the particular genus of the nematode but the isolates of the particular nematode and the temperature conditions during growing that influenced the efficacy of these two nematodes against sciarids and phorids infesting white button and oyster mushrooms. These interpretations are supported by Kim *et al.* (2001) who reported *S. carpocapsae* to be more effective than *H. bacteriophora* against *Lycoriela mali* infesting *P. ostreatus* at 25^{o}C than at 20^{o}C. Grewal *et al.* (1993) found strains ScP and SN of *S. carpocapsae* to be more effective. Number of larval instar treated, inoculum level of the nematode and cropping stage at the time of nematode inoculation also seemed to influence the efficacy of these entomophagous nematodes as indicated by Kim *et al.* (2001) who observed 3rd and 4th instars to be more susceptible than the second instar and Nickle and Contelo (1991) who reported minimum fly emergence with 620 IJs of *S. feltiae* per 100.0 cm^2 of compost. Richardson (1987) found casing treatments to be more effective than spawning treatments. Not much work has earlier been dedicated to management of mushroom flies by using Neem bio-pesticides. The only available reference is of

Bhat *et al.* (1998), who while studying the efficacy of various Neem formulations against insect pests of oyster mushroom found Rakshak @0.15% to be most effective resulting into lowest fruit body infestation.

The inference conceived from present piece of research implies that both the cultivated mushrooms i.e. white button and oyster mushrooms play the most suitable host to dipteran sciarid flies particularly *Sciara* sp. This fly owes its wide distribution in the mushroom units of the state to the unhygienic conditions prevailing in and around most of the farms and the odor emitting from the fermenting compost in open that attracts these pests in large numbers. Once in the farm, the temperature and relative humidity conditions required for mushroom growing are highly conducive for insect multiplication as well. This synchronization of the temperature and humidity between the pest and the host makes the presence of this pest in the farms the year round affair in variable numbers depending upon the environmental conditions as well as the conditions of growing crop. A small developmental phase of maximum of 19 days, high fecundity of 25-60 eggs and voracious feeding by all the larval instars on mycelium as well as fruit bodies resulting into appreciable mycelial devitalization and unhealthy sporocarps necessitate the management of *Sciara* sp. Looking into the unique features of cultivation, extreme sensitivity of the crop and its fresh consumption immediately after harvest makes it imperative to use crop friendly and consumer friendly measures of insect management. The Neem pesticides and entomopathogenic nematodes tried against the insect pests of most commonly cultivated mushrooms during present investigations constitute some of the significant measures that can be taken to control the insect pests. In addition, prophylactic measures and other eco-friendly methods like possible use of certain bacterial bioagents and some antagonistic plants/their products need to be exploited for the management of insect pests in mushrooms.

CHAPTER-6

SUMMARY AND CONCLUSION

The research explorations revealed in the prevenient chapters were conducted to apprise with faunistic affiliation of different group of insects with cultivated edible mushrooms and the impact their presence had, on this highly sensitive crop. All the nine mushroom growing districts of Himachal Pradesh were covered during the survey of various mushroom units growing *Agaricus bisporus* (white button mushroom) and /or *Pleurotus sajor* caju (Oyster mushroom). Insects belonging to two Orders viz. Diptera and Coleoptera were conspicuous by their presence/ abundance in the farms; their population count relating to the conditions of hygiene in and around the cultivation area. Among dipterans, the flies belonging to Family Sciaridae (*Sciara* sp.) and Phoridae (*Megaselia* sp.) registered their presence. Interestingly, *Sciara* sp. recorded for the first time from mushrooms in the country was very prevalent as it infested both the mushrooms under reference in distantly located farms of various districts under survey, working under unhygienic make shift rooms. In most of the locations, the fly count was above the threshold level. With such a high incidence and practically no previous information regarding the status of this insect in mushroom cultivation under Indian conditions available, it was felt worth to select *Sciara* sp. as test insect for further detailed investigations. In addition to faunistic incidence, morphometrics, seasonal abundance and biology of *Sciara* sp. on both the mushrooms was studied. Experiments were conducted to assess the nature of damage, relative susceptibility and damaging potential of the test insect. Symptoms produced as a result of *Sciara* infestation were studied in detail. Also, biomanagement of the test insect by using Neem Seed Kernel Extract, Neem Seed Kernel Powder and entomopathogenic nematodes viz. *Steinernema carpocapsae* and *Heterorhabditis indica* on *A. bisporus* as well as *P. sajor caju* was explored.

Faunistic studies revealed the incidence of insects like *Megaselia* sp., *Sciara* sp. (Order Diptera), *Cyllodes indicus*, *Scaphisoma nigrofasciatum*, *Spondotriplax pallidipes* and *Staphylinus* sp. (Order Coleoptera) in most of the mushroom farms barring purposely built commercial units working under controlled conditions. All these insects except *S. pallidipes* which prevailed only in oyster mushroom infested both *A. bisporus* and *P. sajor caju*. The most frequently occurring dipteran was *Sciara* sp. recorded from all the farms run by marginal farmers. *Megaselia* sp. on the other hand was not as frequent and prevailed only in two districts in low counts. This unreasonably low prevalence of this otherwise known insect of common occurrence could possibly be due to the cool climatic conditions prevailing during the period of survey as *Megaselia* sp. preferred comparatively higher temperatures. Beetles, though, of common occurrence did not prevail as frequently and in as high numbers as flies. The beetle of most common abundance in *A. bisporus* was *Staphylinus* sp. but it was *S. nigrofasciatum* that occurred most frequently in *P. sajor caju*. *Cyllodes indicus* reported for the first time from mushrooms infested *A. bisporus* as well as *P. sajor caju*. Another beetle *S. pallidipes,* also a new record, was found damaging oyster sporocarps. Besides, two genera belonging to newly separated Class Collembola viz. *Achorutes armatus* and *Lepidocyrtus cyaneus* were also encountered in the cool, damp and dark vicinities of cropping rooms in most of the units. Of these, *A. armatus* prevailed more commonly than *L. cyaneus*. Another class of Arthropoda i.e. mites (Acarina) also frequented the mushroom beds, sometimes in enormous numbers. However, their presence/absence only was marked. In overall scenario, though, both mushrooms were highly susceptible to various insect pests, oyster mushroom was more prone than button mushroom. Though, nature of damage of flies, beetles and spring tails was different, all of them caused appreciable damage and spectacular visual symptoms. In addition, these insects also acted as disseminators of nematode and mite pests which had strong phoretic tendencies and when in contact with insects, they often clinged to their legs, thorax and abdomen. Beetles were more efficient carriers than flies.

Observations recorded on the seasonal abundance of *Sciara* sp. for two years revealed the presence of insect in the farm throughout the year in varying numbers. Nil to negligible population was recorded in July-September. The population picked up thereafter to reach the its maximum in March – May.

The eggs of *Sciara* sp. collected from mycelium for biological studies were minute, smooth, oval, dirty white with percent viability of 90.0 per cent. The freshly hatched maggots were cylindrical and had shining black head. These maggots voraciously fed on the mycelial sap and in the process severed its thickened attachments thus hampering further mycelial spread. The larval period lasted for 13-14 days in which four larval instars were recorded. The matured maggots turned into non-feeding, obtect pre-pupae; this period lasted for one to three days. Newly formed pupae were dirty white and changed their color through yellowish brown to deep grey by maturity and had distinct compound eyes. The newly emerged flies were grayish black with filiform antennae having fourteen annuli in the flagellum; typical sciarid wings, long legs with contiguous coxae and elongated abdomen. Abdomen of male fly was slender and shorter with terminal claspers and that of female fly was swollen and terminated into pointed ovipositor. The development cycle from egg to adult emergence in *A. bisporus* (at 23±1^{o}C) was completed averagely in 13.7 days but in *P. sajor caju* (at 26±1^{o}C) it was delayed by three days to complete in 16.7 days. Copulation under captive conditions was observed for the first time. Mating period lasted for four to eight minutes and ovipositing by female started after 15-26 hours of mating. Eggs were laid singly or in clusters of eight to ten. Average fecundity was 29-58 eggs/female. In natural population females were more in number than males and they lived longer than males.

Relative susceptibility of two test mushrooms to *Sciara* sp. assessed under laboratory conditions signified similar role of insect as mycelial inhibitor even at low inoculum level. Two maggots inoculated on fully impregnated mycelia of *A. bisporus and P. sajor caju* incurred mycelial depletion of 12.3 and 14.9per cent respectively as compared to corresponding inhibition of 47.9 per cent and 48.7 per cent by 16 maggots within ten days of inoculation. Damage potential of *Sciara* sp. when worked out under *in vivo* conditions demonstrated progressive increase in mycelial depletion corresponding to increase in inoculum level at all inoculation times. The insect multiplied freely on both the mushrooms; its maggots voraciously feeding on the mycelium as well as pin heads and fruiting bodies and developing in to adults through prepupal and pupal phase that started hovering over the cropping bags. All the developmental stages were observed during analysis of compost/casing soil samples collected from experimental bags. The per cent sporophore yield decline was influenced by the factors like initial inoculum level, mushroom species involved and the cropping stage at which insect infested the crop. Oyster mushrooms were found to be more susceptible as they suffered higher sporophore yield losses than button mushrooms at corresponding levels of inocula and stages of infestation. Under natural conditions also, the former harbored more number of insects than the latter. Maximum yield loss to the tune of 56.7 per cent suffered by *A. bisporus* was lower to the corresponding loss of 63.1 per cent in *P. sajor caju*. In addition to reduction in fruit body production, these insects at high populations also disturbed the flush pattern and shortened the economically viable crop duration.

The maggots sucked the mycelial sap of one hyphal cell after another and in the process restricted the uniform impregnation of the mycelium. The maggots caused most serious damage to newly emerging primordia which turned brown, water soaked or leathery and finally dried without developing into the fruiting bodies. Tunneling by the maggots was pronounced in sporophores of both the mushrooms. The infested sporocarps of oyster mushroom turned hollow, spongy and had a drooping tendency.

Among the bioagents like Neem preparations (NSKE and NSKP) and entomophagous nematodes (*Steinernema carpocapsae* and *Heterorhabditis indica*) tried for the management of *Sciara* sp. though, sporophore production improved significantly in most of the treatments barring those receiving NSKP in lone application as compared to untreated insect inoculated control it was not at par with untreated uninoculated control in most of them. Inoculation of *H. indica*@10^5 IJs per 10 Kg substrate was adjudged the best treatment in both the mushrooms as the sporophore yields in this treatment was recorded to be statistically similar to untreated uninoculated control.

Conclusively, the insect pest fauna associated with two mushrooms is of great implication as they cause drastic qualitative as well as quantitative damage to this highly susceptible commodity. Among these, *Sciara* sp. is a serious hazard because of its wide distribution, low economic threshold level and high damage potential incurring appreciable yield losses. Prophylaxis by maintaining hygiene and management of these insects using crop friendly and environment friendly measures needs to be exploited further in a systematic way.

CHAPTER-7

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* Original not seen

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Title of Thesis	:	Studies on incidence, pathogenicity, biology and biomanagement of insect pests associated with cultivated mushrooms
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Admission Number	:	H-2006-4-D
Major Advisor	:	Dr. Mrs. Anju S. Khanna
Major Field	:	Entomology and Apiculture
Minor Field(s)	:	i) Mycology and Plant Pathology ii) Microbiology
Degree Awarded	:	Ph.D. (Entomology and Apiculture)
Year of Award of Degree	:	2010
No. of pages in Thesis	:	138+XI
No. of words in Abstract	:	411

ABSTRACT

The present piece of research entitled “Studies on incidence, pathogenicity, biology and biomanagement of insect pests associated with cultivated mushrooms” was conducted to acquaint with the insect pest fauna of two commercially cultivated mushrooms viz. *Agaricus bisporus* and *Pleurotus sajor caju*. Abundance of insects belonging to orders Diptera and Coleoptera was recorded in both the mushrooms. In addition, spring tails and mites were also of common occurrence. Two genera of flies belonging to families Sciaridae and Phoridae registered their presence in two mushrooms. Of these, the most prevalent grayish black small fly with filiform antennae, typical sciarid wings, long legs with contiguous coxae and elongated abdomen, was identified as *Sciara* sp. (Diptera: Sciaridae). Looking into its wide distribution in distantly located units, this insect was selected as test insect for further studies. *Megaselia* sp. (Diptera: Phoridae) was observed in two locations only. Incidence of four genera of beetles viz., *Cyllodes indicus*, *Scaphisoma nigrofasciatum*, *Staphylinus* sp. and *Spondotriplax pallidipes* was also recorded. Of these, former three infested both the mushrooms but the last one was found to be present in *P. sajor caju* only. Interestingly, despite its near cosmopolitan distribution in mushroom units of the state *Sciara* sp. has been reported for the first time in mushrooms from Himachal Pradesh. Similarly, *S. pallidipes* has never been recorded earlier from mushrooms in the world. Maggots of *Sciara* sp. and grubs as well as adults of the beetles fed voraciously on the mycelium and fruit bodies of the mushrooms which were highly susceptible to their menace. In addition, these insects also acted as carriers and disseminators of phoretic nematodes and mites. Natural population of *Sciara* sp. comprised of more number of females than males, former living longer than later. The peak population of *Sciara* was observed from March to May under Solan conditions. The developmental phase of *Sciara* from egg to adult was completed in 13.7 days in button mushroom as compared to 16.7 days in oyster mushroom. This fly inflicted heavy quantitative and qualitative yield losses to *A. bisporus* and *P. sajor caju*; later being more susceptible than former. Quantum of damage was influenced by the factors like initial inoculum level, mushroom species involved and cropping stage at which infestation occurred. Infested fruit bodies wore unhealthy appearance. Among the various bioagents tested for their efficacy against the fly, the application of entomopathogenic nematode *Heterorhabditis indica* and/or Neem Seed Kernel Extract (NSKE) improved the sporophore yields of both the mushrooms as compared to control.

Signature of the Major Advisor **Signature of the Student**

Countersigned

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Analysis of Variance (ANOVA)

ANOVA for Table 9

Source	D.F.	S.S.	M.S. S.	F-cal
Larvae (L)	4	1387.18	346.79	495.20
Days (D)	4	17360.656	4340.16	6197.47
L x D	16	442.41	27.65	39.48
Error	50	35.02	0.70	
Total	74	19225.27		

ANOVA for Table 10

Source	D.F.	S.S.	M.S. S.	F-cal
Larvae (L)	4	1479.80	369.50	827.28
Days (D)	4	18150.13	4537.53	10146.82
L x D	16	427.63	26.73	59.77
Error	50	22.36	0.45	
Total	74	20079.92		

ANOVA for Table 11

Source	D.F.	S.S.	M.S. S.	F-cal
Larvae (L)	2	583.42	291.71	119.91
Days (D)	3	1042.27	347.43	142.82
L x D	6	437.72	72.95	29.99
Error	24	58.39	2.43	
Total	35	2121.80		

ANOVA for Table 12

Source	D.F.	S.S.	M.S. S.	F-cal
Larvae (L)	2	134.76	67.38	9.39
Days (D)	3	1947.61	649.20	90.44
L x D	6	71.65	11.94	1.66
Error	24	172.27	7.18	
Total	35	2326.29		

ANOVA for Table 13

Source	D.F.	S.S.	M.S. S.	F-cal
Larvae (L)	2	222.60	111.30	15.13
Days (D)	3	2290.12	763.37	103.78
L x D	6	136.69	22.78	3.09
Error	24	176.53	7.35	
Total	35	2825.95		

ANOVA for Table 14

Source	D.F.	S.S.	M.S. S.	F-cal
Larvae (L)	2	144.05	72.02	10.47
Days (D)	3	1876.70	625.56	90.95
L x D	6	62.59	10.43	1.52
Error	24	165.08	6.87	
Total	35	2248.42		

ANOVA for Table 15

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae (L)	2	18.53	9.27	218.41
Intervals (I)	3	19.56	6.52	153.68
L x I	6	2.46	0.41	9.67
Population (P)	2	12.42	6.21	146.34
L x P	4	6.96	1.74	41.04
I x P	6	1.22	0.20	4.78
L x I x P	12	0.78	0.06	1.52
Error	72	3.05	0.04	
Total	107	64.98		

ANOVA for Table 16

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae (L)	2	3.99	1.99	59.39
Intervals (I)	2	0.69	0.35	10.35
L x I	4	31.13	7.78	231.41
Population (P)	2	7.11	3.56	105.77
L x P	4	1.79	0.45	13.31
I x P	4	0.04	0.01	0.27
L x I x P	8	6.59	0.82	24.51
Error	24	1.82	0.03	
Total	80	53.17		

ANOVA for Table 17

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae (L)	2	18.53	9.27	218.42
Intervals (I)	3	19.56	6.52	153.69
L x I	6	2.46	0.41	9.68
Population (P)	2	12.42	6.21	146.35
L x P	4	6.96	1.74	41.05
I x P	6	1.22	0.20	4.79
L x I x P	12	0.78	0.07	1.53
Error	72	3.05	0.04	
Total	107	64.98		

ANOVA for Table 18 (1st week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	63857.67	21285.89	6.68
Error	8	25489.33	3186.17	
Total	11	89347.00		

ANOVA for Table 18 (2nd week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	126038.88	42012.96	38.87
Error	8	8646.00	1080.75	
Total	11	134684.88		

ANOVA for Table 18 (3rd week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	42578.938	14192.979	30.938
Error	8	3670.000	458.750	
Total	11	46248.938		

ANOVA for Table 18 (4th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	39730.91	13243.64	7.49
Error	8	14148.00	1768.50	
Total	11	53878.91		

ANOVA for Table 18 (5th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	7128.92	2376.31	5.04
Error	8	3776.00	472.00	
Total	11	10904.92		

ANOVA for Table 18 (6th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	13089.58	4363.19	4.90
Error	8	7120.67	890.08	
Total	11	20210.25		

ANOVA for Table 19 (1st week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	23833.29	7944.43	2.78
Error	8	22833.33	2854.17	
Total	11	46666.63		

ANOVA for Table 19 (2nd week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	161722.88	53907.63	43.78
Error	8	9850.00	1231.25	
Total	11	171572.88		

ANOVA for Table 19 (3rd week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	46244.27	15414.76	31.55
Error	8	3908.67	488.58	
Total	11	50152.94		

ANOVA for Table 19 (4th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	51574.00	17191.33	27.48
Error	8	5004.00	625.50	
Total	11	56578.00		

ANOVA for Table 19 (5th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	11472.92	3824.31	4.98
Error	8	6150.00	768.75	
Total	11	17622.92		

ANOVA for Table 19 (6th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	11639.58	3879.86	4.88
Error	8	6366.67	795.83	
Total	11	18006.25		

ANOVA for Table 20 (1st week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	36956.27	12318.76	3.15
Error	8	31266.67	3908.33	
Total	11	68222.94		

ANOVA for Table 20 (2nd week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	153241.63	51080.54	30.27
Error	8	13500.00	1687.50	
Total	11	166741.63		

ANOVA for Table 20 (3rd week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	52875.61	17625.20	19.50
Error	8	7229.33	903.67	
Total	11	60104.94		

ANOVA for Table 20 (4th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	68165.66	22721.89	20.30
Error	8	8954.00	1119.25	
Total	11	77119.66		

ANOVA for Table 20 (5th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	26756.25	8918.75	14.81
Error	8	4816.67	602.08	
Total	11	31572.91		

ANOVA for Table 20 (6th week)

Source	D. F.	S. S.	M. S. S.	F-cal
Larvae	3	21575.00	7191.67	39.23
Error	8	1466.67	183.33	
Total	11	23041.66		

ANOVA for Table 22

Source	D.F.	S.S.	M.S. S.	F-cal
Inoculation time (I)	2	60.734	30.367	9.876
Larvae (L)	3	2531.750	843.917	274.456
I x L	6	171.984	28.664	9.322
Error	24	73.797	3.075	
Total	35	2838.266		

ANOVA for Table 23

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae (L)	2	25.75	12.87	263.05
Intervals (I)	3	29.90	9.97	203.68
L x I	6	3.35	0.56	11.40
Population (P)	2	11.85	5.92	121.05
L x P	4	6.64	1.66	33.93
I x P	6	0.69	0.11	2.34
L x I x P	12	1.05	0.09	1.79
Error	72	3.52	0.05	
Total	107	82.75		

ANOVA for Table 24

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae (L)	2	21.51	10.76	199.14
Intervals (I)	2	6.75	3.73	62.46
L x I	4	1.19	0.29	5.54
Population (P)	2	10.30	5.15	95.33
L x P	4	5.72	1.43	26.48
I x P	4	0.07	0.02	0.32
L x I x P	8	0.57	0.07	1.32
Error	54	2.92	0.05	
Total	80	49.03		

ANOVA for Table 25

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae (L)	2	31.78	15.89	626.31
Intervals (I)	3	34.72	11.57	456.08
L x I	6	4.89	0.81	32.10
Population (P)	2	12.58	6.29	247.91
L x P	4	6.31	1.58	62.12
I x P	6	0.45	0.08	2.94
L x I x P	12	0.73	0.06	2.39
Error	72	1.83	0.03	
Total	107	93.28		

ANOVA for Table 26 (1st week)

Source	**D.F.**	**S. S.**	**M.S. S.**	**F-cal**
Larvae	3	286141.34	95380.45	60.80
Error	8	12550.66	1568.83	
Total	11	298692.00		

ANOVA for Table 26 (2nd week)

Source	**D.F.**	**S. S.**	**M.S. S.**	**F-cal**
Larvae	3	1184139.38	394713.13	65.95
Error	8	47883.63	5985.45	
Total	11	1232023.00		

ANOVA for Table 26 (3rd week)

Source	**D.F.**	**S. S.**	**M.S. S.**	**F-cal**
Larvae	3	537550.00	179183.33	147.02
Error	8	9750.00	1218.75	
Total	11	547300.00		

ANOVA for Table 26 (4th week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	289074.97	96358.32	54.80
Error	8	14066.66	1758.33	
Total	11	303141.63		

ANOVA for Table 26 (5th week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	194072.91	64690.97	20.87
Error	8	24800.00	3100.00	
Total	11	218872.91		

ANOVA for Table 27 (1st week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	7508.00	2502.67	1.23
Error	8	16334.00	2041.75	
Total	11	23842.00		

ANOVA for Table 27 (2nd week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	382922.34	127640.78	88.15
Error	8	11583.66	1447.96	
Total	11	394506.00		

ANOVA for Table 27 (3rd week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	192222.328	64074.19	54.63
Error	8	9383.67	1172.96	
Total	11	201606.00		

ANOVA for Table 27 (3rd week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	192222.328	64074.19	54.63
Error	8	9383.67	1172.96	
Total	11	201606.00		

ANOVA for Table 27 (4th week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	103139.59	34379.86	39.20
Error	8	7016.66	877.08	
Total	11	110156.25		

ANOVA for Table 27 (5^{th} week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	87841.66	29280.56	10.66
Error	8	21983.34	2747.92	
Total	11	109825.00		

ANOVA for Table 27 (Total yield)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	5	34326092.00	6865218.50	107.30
Error	18	1151672.00	63981.78	
Total	23	35477764.00		

ANOVA for Table 28 (1^{st} week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	530188.69	176729.56	121.03
Error	8	11684.31	1460.54	
Total	11	541873.00		

ANOVA for Table 28 (2^{nd} week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	1152542.00	384180.66	543.97
Error	8	5650.00	706.25	
Total	11	1158192.00		

ANOVA for Table 28 (3^{rd} week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	616683.31	205561.11	254.30
Error	8	6466.69	808.34	
Total	11	623150.00		

ANOVA for Table 28 (4^{th} week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	326541.69	108847.23	316.65
Error	8	2750.00	343.75	
Total	11	329291.69		

ANOVA for Table 28 (5^{th} week)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	3	210416.67	70138.89	39.42
Error	8	14233.33	1779.17	
Total	11	224650.00		

ANOVA for Table 28 (Total yield)

Source	D.F.	S. S.	M.S. S.	F-cal
Larvae	5	34326092.00	6865218.50	107.30
Error	18	1151672.00	63981.78	
Total	23	35477764.00		

ANOVA for Table 30

Source	D.F.	S.S.	M.S. S.	F-cal
Inoculation time (I)	2	143.94	71.97	21.68
Larvae (L)	3	2235.30	745.01	224.49
I x L	6	18.81	3.13	0.95
Error	24	79.66	3.32	
Total	35	2477.70		

ANOVA for Table 31

Source	D.F.	S.S.	M.S. S.	F-cal
Time Interval (I)	4	1387.18	346.79	495.20
Treatments (T)	14	17360.656	4340.16	6197.47
I x T	56	442.41	27.65	39.48
Error	50	35.02	0.70	
Total	74	19225.27		

ANOVA for Table 32

Source	D.F.	S.S.	M.S. S.	F-cal
Time Interval (I)	4	1482.63	370.66	398.52
Treatments (T)	14	16856.23	1204.36	5875.24
I x T	56	589.75	27.65	10.53
Error	50	35.02	0.70	
Total	74	18939.63		

ANOVA for Table 34

Source	D.F.	S.S.	M.S. S.	F-cal
Time Interval (I)	1	69193.19	69193.19	5608.51
Treatments (T)	15	1865.73	124.38	10.08
I x T	15	91.53	6.10	0.49
Error	64	789.58	12.37	
Total	95	71939.95		

ANOVA for Table 35

Source	D.F.	S.S.	M.S. S.	F-cal
Time Interval (I)	1	77231.08	77231.08	5285.96
Treatments (T)	15	2245.45	149.70	10.25
I x T	15	241.78	16.12	1.10
Error	64	935.08	14.61	
Total	95	80653.39		

ANOVA for Table 36

Source	D.F.	S.S.	M.S. S.	F-cal
Treatments (T)	15	14924.33	994.96	5.67
Error	32	5612.67	175.40	
Total	47	20537.00		

ANOVA for Table 37

Source	D.F.	S.S.	M.S. S.	F-cal
Treatments (T)	15	3147.33	209.82	1.65
Error	32	4058.67	126.83	
Total	47	7206.00		

ANOVA for Table 38

Source	D.F.	S.S.	M.S. S.	F-cal
Treatments (T)	15	26303.94	1753.60	11.90
Error	32	4714.00	147.31	
Total	47	31017.94		

ANOVA for Table 40

Source	D.F.	S.S.	M.S. S.	F-cal
Time Interval (I)	4	5659.20	1414.80	794.32
Treatments (T)	14	16082.25	1148.73	644.94
I x T	56	1280.52	22.87	12.84
Error	150	267.17	1.78	
Total	224	23289.14		

ANOVA for Table 41

Source	D.F.	S.S.	M.S. S.	F-cal
Time Interval (I)	4	4875.65	1218.91	658.23
Treatments (T)	14	15074.26	1076.73	548.59
I x T	56	1132.42	20.22	10.54
Error	150	210.81	1.41	
Total	224	21293.14		

ANOVA for Table 43

Source	D.F.	S.S.	M.S. S.	F-cal
Treatments (T)	15	62948.00	4196.53	7.28
Error	32	18450.00	576.56	
Total	47	81398.00		

ANOVA for Table 44

Source	D.F.	S.S.	M.S. S.	F-cal
Treatments (T)	15	6095.00	406.33	1.55
Error	32	8367.00	261.47	
Total	47	14462.00		

ANOVA for Table 45

Source	D.F.	S.S.	M.S. S.	F-cal
Treatments (T)	15	81731.25	5448.75	7.68
Error	32	22700.00	709.38	
Total	47	104431.25		

CURRICULUM VITAE

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Certificate/ degree	**Class/ grade**	**Board/ University**	**Year**
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10+2	First	H.P.B.S.E., Dharamshala	1999
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Whether sponsored by some state/ Central Govt./Univ./SAARC : NA

Scholarship/ Stipend/ Fellowship, any other financial assistance received during the study period : University Merit Scholarship

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