

**STUDIES ON HOUSE FLY INFESTATION IN
POULTRY FARMS WITH EMPHASIS ON ITS
MANAGEMENT**

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VETERINARY COLLEGE, BANGALORE-24
KARNATAKA VETERINARY, ANIMAL AND FISHERIES
SCIENCES UNIVERSITY, BIDAR**

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**STUDIES ON HOUSE FLY INFESTATION IN
POULTRY FARMS WITH EMPHASIS ON ITS
MANAGEMENT**

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By

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SCIENCES UNIVERSITY, BIDAR
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CERTIFICATE

This is to certify that the thesis entitled "*Studies on house fly infestation in poultry farms with emphasis on its management*" submitted by Ms. ARCHANA, M., I.D. No. DVHK-1313 in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY in VETERINARY PARASITOLOGY** of the **Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar**, is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associate ship, fellowship or other similar titles.

Bangalore

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Affectionately Dedicated to
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LIST OF ABBREVIATIONS / ACRONYMS

BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
bp	Base pair
mm	Millimeter
cm	Centimeter
°C	Degree Celsius
DNA	Deoxyribonucleic acid
dNTP	2'-deoxyribonucleoside-5' triphosphate
EC	Electrical Conductivity
EDTA	Ethylene Diamine Tetra Acetic acid
EO	Essential oil
EPNs	Entomopathogenic nematodes
<i>et al.</i>	<i>et alia</i>
<i>etc.</i>	<i>et cetera</i>
Fig.	Figure
Gm (s)	Gram (s)
g	Gram
Hr (s)	Hour (s)
IPM	Integrated pest management
LD	Lethal dose
µl	Microliter
mg	Milligram
ml	Milliliter
min	Minutes
M	Molar
NaCl	Sodium Chloride
NCBI	National Centre for Biotechnology Information
NFW	Nuclease Free Water
NTC	No Template Control

%	Per cent
PCR	Polymerase Chain Reaction
pm	Pico moles
rpm	Revolution per minute
sec	Seconds
spp	Species
TBE	Tris Borate EDTA
TE	Tris EDTA
U	Unit
UV	Ultra Violet
V	Volts
v/v	volume/volume
w/v	weight/volume
CO1	Cytochrome oxidase 1
F	Forward
R	Reverse
Fig	Figure
DAT	Days after treatment
IJs	Infective juveniles
LC	Lethal concentration
cm ³	Cubic centimeter
PIR	Percent inhibition rate
ppm	Parts per million
ER	Effective repellence
DE	Diatomaceous earth
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy

Introduction



I. INTRODUCTION

India's poultry industry represents a major success story. It is one of the fastest growing segments of the agricultural sector today in India with annual growth rates of 5.57 percent and 11.44 percent in egg and broiler production, respectively. Agricultural production has been rising at the rate of 1.5- 2 percent per annum over the past two to three decades while poultry production has been rising at the rate of 8-10 percent per annum, with an annual turnover of US\$ 7500 million. The total poultry population in India is 729.2 million, which is 12.39 percent higher than the previous census (Livestock census, 2012). The sector is providing direct or indirect employment to 6.5 million people. It accounts for about one percent of India's GDP and 11.70 percent of the GDP from the livestock sector. Livestock population of India is among the highest in the world, it contributes approximately 4 % to GDP and 27 % to agricultural GDP (Ali, 2015). South India accounts for majority of total poultry production and consumption in the country (Abroader Consultancy India Pvt Ltd, 2015). The higher growth places India at 3rd position after China and USA with a production of 59.8 billion eggs and 5th position after USA, China, Brazil, and Mexico in broiler production (Central Avian Research Institute, 2011).

Fly menace in poultry farms is one of the significant problems. Several species of flies are found associated with poultry production facilities such as houseflies and their relatives; flesh flies, blow flies, bottle flies, filter flies, soldier flies and vinegar or fruit flies. These flies cause annoyance, discomfort and are also harmful to human and animal health. These flies are usually scavengers in nature and are capable of transmitting many

diseases to human and animals. In view of the severity of the problem, these flies need to be studied and effective control measures have to be implemented. There is very scanty information on the prevalence of different flies in poultry farms in most parts of India including Karnataka. A detailed study is essential to know the different fly species. In view of this scenario and lack of seasonal prevalence study in Karnataka, the present study was undertaken in Bangalore district to observe the prevalence of flies in poultry farms under different managemental conditions.

Insects are usually identified by morphological methods but it is tedious, time-consuming, needs a specialist, holotype comparison, difficult to identify large number of insects in a short period of time and requires taxonomic expertise. The morphological similarity in females of certain species complex of insects is a challenge for identification. Therefore, molecular method of identification is being advocated for identification. Molecular method of identification is faster and has increased sensitivity and specificity. One such molecular method is DNA barcoding (Kim *et al.*, 2012; Rivera and Currie, 2009; Hebert *et al.*, 2003) and this approach was employed for confirmation of morphologically identified insects.

Among flies, which are prevalent in poultry farms, housefly *Musca domestica* L (Diptera: Muscidae) is a major pest (Harikrishnan, 2011a). It represents about 90% of the total fly species associated with poultry manure, especially in caged-layer operations. Accumulated poultry manure can be highly suitable for fly breeding. The housefly is also a major domestic, medical pest and is regarded as a public health hazard, predominantly in parts of the world where sanitary and hygienic conditions are poor. They can cause

flyspecking problems by regurgitated and faecal material on eggs as well as windows, walls of buildings, equipment, egg trays, lights and egg racks leading to black marks due to their depositions of excreta leading to spoiling of eggs, defacing cages and reduced illumination of lights (Muniyellappa, 2010; Iqbal *et al.*, 2014). High population density of *M. domestica* in poultry farms spoils food and causes annoyance to farm employees. It also causes annoyance to neighbouring rural non-farming communities and often leads to poor community relations and potential litigation.

Houseflies serve as vectors and reservoirs for food borne diseases as they carry pathogenic organisms in their mouth parts, faeces and other external body parts. They also visit waste materials from garbage or sewage and spread microbes to human and animal food (Barro *et al.*, 2006; Khobdel *et al.*, 2008; Loftin *et al.*, 2014). More than 100 pathogens associated with the housefly cause intestinal diseases in humans and animals such as protozoa (*Giardia*, *Entamoeba* etc), bacteria (*Escherichia coli*, *Salmonella* etc), helminths (round worms, hookworms, pinworms and tapeworms), fungi, viruses and rickettsial organisms (Barin *et al.*, 2010; Jesus *et al.*, 2004). They act as intermediate hosts for tapeworms of poultry. Avian influenza is spread principally by contaminated shoes, clothes and equipment. The virus has been isolated from adult houseflies, proving potentiality of transmission by houseflies (Rutz, 2000). Accidentally when larvae of *M. domestica* are swallowed in food material they can survive in the human gut and causes intestinal myiasis with symptoms of pain, nausea and vomiting (Shekhawat *et al.*, 1993; Sehgal *et al.*, 2002; Achra *et al.*, 2014).

In a view of the severity of the problem as a part and the vectoral role, it is important to control *M. domestica*. Management of these pests relies upon the integration of mechanical, cultural and chemical approaches. One of the main method to control flies in poultry farms is by means of chemical insecticides with many limitations. Insecticides also increase the mortality of non-target organisms when mixed in the manure and causes destruction of other beneficial arthropods which act as natural enemies of the housefly. It also persists in the environment and can be biomagnified through the food chain, risk of residue in the eggs and in the meat of the poultry (Kamaraj *et al.*, 2011). Therefore any attempt to scale down the use of chemical insecticides is welcome considering the safety to flora and fauna of the environment. Due to environmental and regulatory pressures, research towards developing alternative pest control measures are warranted (Tomerlin, 2000). Therefore, identification of novel effective alternative strategies to manage this important pest by using more eco-friendly methods such as use of biological agents (bacteria, virus, fungi, entomopathogenic nematodes etc), semiochemicals and phytochemicals are of paramount importance for the development of successful control of housefly.

The use of chemical insecticides for control of housefly has many disadvantages and is economically not viable in larger scale. One of the methods for exploitation in housefly control is by its application through semiochemicals. The toxic bait preparation with a pheromone as attractant and a suitable insecticide has been mooted by Chapman *et al.* (1998). A new mode of application of insecticides was therefore evaluated in the present study by semiochemicals- “Attraction and kill” strategies by combining an

attractant (semiochemicals) with an insecticide in a gel matrix for slow release for control of flies.

Biological pest control has been developed primarily by entomologists and involves the use of living natural enemies to control pest species. In pest management, biological control usually refers to the action of parasitoids, predators or pathogens on a pest population which reduces its numbers below a level causing economic injury. The various biological agents in housefly control include the use of virus, bacteria, fungi, nematodes, parasites, parasitoids and predatory insects.

Botanical pesticides are important alternatives to minimize or replace the use of synthetic pesticides as they possess an array of properties including insecticidal, antifeedant, repellent and oviposition deterrent, growth regulatory and anti vector activities (Isman, 2006). It is reported that essential oils possess fumigant insecticide properties due to the presence of acetyl cholinesterase inhibition and octopaminergic action. As this target site is not shared with mammals, most essential oil chemicals are relatively non toxic to mammals and fish in toxicological tests and meet the criteria for reduced risk pesticides (Enan, 2001). These botanicals fit perfectly well into housefly management programme due to their safety to non target organisms and the environment. Few studies are at preliminary stage for investigating the effect of botanical pesticides on housefly. Therefore, in the present study some of the essential oils such as Curcumin, neem, Sweet basil and Cumin seed has been evaluated against eggs, larvae, pupae and adult houseflies.

Diatomaceous earth (DE) is pure amorphous silicon dioxide made up of fossilised diatoms; it acts as an insecticide by absorption of epicuticular lipids and fatty acids, leading to desiccation in arthropods (Jean *et al.*, 2015). DE is basically a lethal dust lined with microscopic razor sharp edges, as insects cross over the powder, the edges cut through the insect's protective exoskeleton and causes the insect to dehydrate from the outside. Diatomaceous earth is highly absorptive amorphous silica that can reduce the moisture in poultry litter making it a less attractive environment for flies to breed in and reduce the suitability for larval development.

Considering all the aspects of fly problem in poultry farms with particular reference to houseflies the present study was undertaken with following objectives:

- A. Prevalence and diversity of fly infestation in poultry farms
- B. Evaluation of new modes of application with semiochemicals
- C. Evaluation of biopesticides against fly control
- D. Evaluation of biocontrol agents such as entomopathogenic nematodes for fly control
- E. Evaluation of agripower diatomite (diatomaceous earth) for control of flies

Review of Literature



II. REVIEW OF LITERATURE

Houseflies serve as vectors and reservoirs for food borne diseases as they carry pathogenic organisms in their mouth parts, faeces and other external body parts. They also visit waste materials from garbage or sewage and spread microbes to human and animal food. More than 100 pathogens associated with the housefly cause intestinal diseases in humans and animals such as protozoa, bacteria, helminths, fungi, viruses and rickettsial organisms (Barin *et al.*, 2010; Jesus *et al.*, 2004). They act as intermediate hosts for tapeworms of poultry. Avian influenza is spread principally by contaminated shoes, clothes, and equipment, the virus has been isolated from adult houseflies, proving potentiality of transmission (Rutz, 2000). Accidentally when larvae of house fly are swallowed in food material they can survive in the human gut and causes intestinal myiasis with symptoms of pain, nausea and vomiting (Achra *et al.*, 2014). In a view of severity of the problem as a part and the vectoral role, it is important to control *M. domestica*. Therefore the following literature has been reviewed with respect to prevalence, morphology, DNA barcoding and different methods of control viz. semiochemical, biopesticides, entomopathogenic nematodes, diatomaceous earth.

2.1 Prevalence and diversity of fly infestation in poultry farms

Fly menace in poultry farms is one of the significant problems. Flies cause annoyance, discomfort and are also harmful to human and animal health. In view of the severity of the problem, these flies need to be studied and effective control measures have to be implemented. As there is very scanty information on the prevalence of different

flies in poultry farms in most parts of India including Karnataka. Therefore the following literature has been reviewed with respect to prevalence.

2.1.1 In India

Rahman (1977) reported the commonly encountered species of *Musca* in Karnataka viz, *Musca vicina*, *M. nebulo*, *M. yerburyi*, *M. sorbens* Wiedemann, 1830 and *M. vetustissima* Walker, 1849 with *M. vicina* being the most commonly encountered housefly. He also reported occurrence of entomopathogenic fungus *Entomophthora muscae* (Cohn) Fresen on housefly in Karnataka.

Valandikar and Hiregoudar (1996) reported the number and species of calliphorid, muscid and sarcophagid flies from different parts of Karnataka. The species of flies recorded were *Chrysomya megacephala*, *C. rufifacies*, *C. bezziana* Villeneuve, 1914, *Lucilia porphyrina* Walker, 1856, *Phaenicia cuprina*, *P. sericata* Meigen, 1826, *Hemipyrelia liguriens* Wiedemann and *Bengalia jejuna* Fabricius, 1787, *M. vicina*, *M. nebulo*, *Sarcophaga dux* Thomson, 1869 and *S. brevicornis* Ho, 1934. The seasonal incidence of calliphorid flies at Bangalore showed peak from June to September followed by a decline in October and November showing that the calliphorid flies were most common during rainy season.

Harikrishnan (2009) listed several kinds of flies common in and around caged layer houses in Namakkal, Tamil Nadu such as housefly *M. domestica* as a major pest species, little housefly, *F. canicularis*. Also reported various biological control agents such as fungal pathogens, parasitoids and predators, botanical pesticides and bacterial pathogens for control of houseflies.

Muniyellappa (2010) listed the common flies on poultry including housefly (*M. domestica*), little housefly (*F. annicularis*) coastalfly (*F. femoralis*), latrinefly (*F. scalaris*), false stable fly (*M. stabulans*), blowflies, fleshflies and filthflies in Karnataka.

Harikrishnan (2011a) listed several kinds of flies common in and around caged layer houses such as housefly *M. domestica*, little housefly *F. canicularis*, black garbage fly *H. aenescens* in Namakkal, Tamil Nadu. The use of various biological control agents such as fungal pathogens, parasitoids and predators, botanical pesticides and bacterial pathogens for control of house flies was highlighted.

Ponnudurai and Harikrishnan (2011) reported the intensity of housefly population in four caged layer poultry houses in relation to seasonal variation. A standard spot card method was used to monitor the housefly population in four commercial farms in Namakkal, Tamil Nadu. Data collected in all the farms from October 2004 to September 2005 showed an extremely high fly population (279 spots/card) in the month of October with onset of North-east monsoon. There after a low fly population was recorded during winter months from December to March first week. On the arrival of summer season, the population again began to increase from March third week and reached maximum numbers in the month of June as 251-spots/card in Namakkal, Tamil Nadu.

Ponnudurai (2013) listed flies prevalent in poultry farms in Namakkal, Tamil Nadu such as house flies as a major pest, also other flies with less importance such as *Chrysomya* and *Fannia*. He also listed various biological control agents such as fungal pathogens, parasitoids and predators, botanical pesticides and bacterial pathogens for control of houseflies.

Divya and Sathe (2015) reported diversity of forensic blowflies (Diptera: Calliphoridae) from Western Ghats in Maharashtra. A total of 22 species belonging to 9 genera have been reported. The important genera were *Silbomyia*, *Calliphora*, *Phumosia*, *Melinda*, *Lucilla*, *Pollenia*, *Tianania*, *Dexopollenia* and *Chrysomya*. *Chrysomya megacephala* was also reported.

Harikrishnan (2015) reported *M. domestica* as a major fly in poultry farms and also reported problems associated with housefly, monitoring of flies and integrated control of house flies in poultry farms.

Sumathi *et al.* (2016) did a comparative study on housefly population monitoring techniques in caged layer poultry houses at Nammakal, Tamil Nadu. Fly monitoring was carried out in Namakkal poultry houses from September 2007 to December 2007. The spot card and baited jug trap methods were used in this study to monitor adult housefly population in two narrow caged poultry houses. Spot cards were tied to stand of cages at 2' above the floor in poultry houses for 24 h. The spot cards were removed and regurgitation and the faecal spots made by the adult flies were counted for spot card index. In baited jug trap methods there were closed buckets (4lt) with four access holes of 5cm diameter in upper third of the bucket, these traps were hung from rafters in poultry houses. Bait comprising 12g each of rice bran and jaggery mixed in 15ml of water and incorporated with either dichlorovos (316.6mg) or spinosad (187.5mg). The baited jug traps were placed for each pesticide and were allowed to be in house for about 24 h. The spot card index was highest in October and November. In baited jug trap method, the maximum fly density was witnessed in the month of October and November for spinosad

and September and November for dichlorovos. Mean number of fly specks/ card/day/spot card and mean number of flies caught per day in baited jug trap differ significantly ($p < 0.01$). The mean number of flies caught/jug trap/day between spinosad baited jug trap and dichlorovos baited jug traps differed significantly ($p < 0.01$).

2.1.2 In different parts of world

Axtell (1970) listed flies associated with the poultry farms in United States viz, housefly *Musca domestica* Linnaeus, 1758, little housefly *Fannia canicularis* Linnaeus, 1761, black garbage fly *Ophyra leucostoma* Wiedemann, black blowfly *Phormia regina* Meigen, 1826, stablefly *Stomoxys calcitrans* Linnaeus, 1758 and green blowflies *Phaenicia* spp. and also listed various integrated fly control programme for caged poultry farms.

Toyama and Ikeda (1976) listed the fly parasites in animal farms including poultry in Leeward and Central Oahu and reported the prevalence of *Musca domestica*, *Stomoxys calcitrans*, *Ophyra chalcogaster* Wiedemann 1984, *Fannia pusio* Wiedemann, 1830, *Phaenicia cuprina* Wiedemann, 1830, *Chrysomya megacephala* Fabricius, 1794, *Chrysomya rufifaces* Macquart, 1842, *Parasarcophaga ruficornis* Fabricius, 1794, *Parasarcophaga argyrostoma* Robineau-Desvoidy, 1830, *Seniorwhitea orientaloidea* Senior-White, 1924, *Volucella obesa* Van DER Wore, 1882, *Eristalis arvorum* Fabricius, 1787 and *Hermetia illucens* Linnaeus.

Hulley (1986) reported the prevalence of flies which breed in manure accumulation in poultry houses in Grahamstown. The different flies reported were *Simoides pachymera* Bezzi, 1915, *Sepsis lateralis* Weidemann, 1830, *Coproica*, *Fannia*

leucosticte Meigen, 1838, *Fannia canicularis*, *F. femoralis* (Stein), *M. domestica*, *Chrysomya putoria* Wiedemann, 1830 and *M.stabulans* Fallen, 1817.

Axtell (1999) listed various flies which are associated with the poultry farms in USA such as housefly (*M. domestica*), little housefly (*F. annicularis*), dumbflies (*H. aenescense* Wiedemann, 1830, *H. capensis* Wiedemann, 1818, *H. ignava* Harris, 1780), black soldierfly, *Hermetia illucens*, *Drosophila replete*, blowflies such as *Lucilia*, *Phaenicia*, *Phormia*, and *Calliphora*. *L. cuprina* and *L. sericata* also sometimes associated with poultry farms. Biting flies such as stablefly, *Stomoxys calcitrans*, *Culex quinquefasciatus* Say, 1823 and *C. pipiens* Linnaeus, 1758. Moth flies such as the meal moth, *Pyralis farinalis* Linnaeus, 1758, mediterranean flour moth, *Anagasta kuehniella* Zeller, 1879 and the Indian meal moth *Plodia interpunctella* Hübner, 1813, cockroaches such as German, *Blattella germanica* Linnaeus, 1767, American, *Periplaneta americanum* Linnaeus, 1758 and Oriental, *Blatta orientalis* Linnaeus, 1758.

Rutz (2000) listed flies associated with manure in USA such as *Musca domestica*, *Fannia canicularis*, *H. aenescens* and small dung flies of the family Sphaeroceridae.

Williams (2010) listed various flies associated with the poultry in USA such as housefly – *M. domestica*, little housefly – *Fannia* sp. black garbagefly – *H. aenescens*, numerous species of blow flies (green or blue bottle flies) and small dungfly – Sphaeroceridae.

Saif *et al.* (2011) listed flies associated with poultry production such as houseflies *M. domestica*, little houseflies *F. canicularis*, blowflies of Calliphoridae, fleshflies-Sarcophagidae, dung flies-Sphaeroceridae and fruit flies-Drosophilidae.

Tucci (2011) listed the most frequent species of flies which are associated with poultry farms in Brazil such as *M. domestica*, *S. calcitrans*, *M. stabulans*, *Fannia* spp, *C. putoria* and *H. illucens*.

Levot (2013) listed flies which are associated with the poultry farms in States of New South Wales viz, *M. stabulans*, *F. canicularis*, *M. domestica*, soldierfly, *H. illuscens*, *S. calcitrans*, *Alphitobius diaperinus* Panzer, 1797 also known as lesser mealworm.

Loftin *et al.* (2014) reported different flies associated with the poultry manure, biology and various control methods for flies in poultry farms.

2.2 Morphological identification

The morphological identification of different flies was carried out with the keys provided by various authors. Therefore the following literature has been reviewed with respect to morphological identification.

White *et al.* (1940) described and provided keys for identification of Calliphorid flies.

Sabrosky (1949) provided the keys for Muscid genus *Ophyra* in the Pacific Region (Diptera) and described the different species of *Ophyra* and their distribution in the Pacific region.

James (1960) provided a key to the subfamilies and genera of soldier flies or stratiomyidae of California and also described the geographical distribution and occurrence of different species.

Lopes (1961) provided a key for identification of the 14 species of flies from Hawaii based mainly on external characters and reported the geographical distribution of these flies.

Van Emden (1965) described and provided keys for identification of muscid flies.

Zumpt (1965) described various morphological features of myiasis producing larvae and adult flies and provided keys for identification.

Fonsseca (1968) described and provided keys for identification of muscid flies.

Huckett (1975) described keys for subfamilies of Muscinae and Stomoxyinae and later the various genera within subfamilies and described various species of Muscinae and Stomoxyinae of California.

Smith (1989) described the collection, rearing, preservation and examination of larvae, biology and morphology of immature stages and also described keys to the suborder of British dipteran larvae and described the keys to British Nematocera, Brachycera and Cyclorrhapha.

Holloway *et al.* (1991) provided keys to identify third-instar larvae of the 9 species of calliphorids causing flystrike and associated with carrion in New Zealand. The species included *Calliphora hilli* Patton, 1925, *C. quadrimaculata* Swederus, 1787, *C.*

sygia Fabricius, 1781, *C. vicina* Robineau-Desvoidy, 1830, *Chrysomya megacephala*, *C. rufifacies*, *Lucilia cuprina*, *L. sericata* and *Xenocalliphora hortona* Walker, 1849.

Walker (1994) provided basic morphological features for identification of all arthropods of humans and domestic animals.

Wells *et al.* (1999) provided a key to 3rd instars of 8 Chrysomyine species reported from or likely to occur in carrion within the continental United States.

Sukontason *et al.* (2003a) described larval morphology of *Chrysomya megacephala* using scanning electron microscopy. The relative thickness of the branches of the posterior spiracular hairs in these species could be used to differentiate them in this developmental stage. In contrast, the hairy appearance of *C. rufifacies* allowed second- and third-instar larvae to be easily distinguished.

Sukontason *et al.* (2003b) described larval ultrastructure of *Parasarcophaga dux* (Diptera: Sarcophagidae) using scanning electron microscopy. Anterior and posterior spiracles are important features used to differentiate between other sarcophagids. Each anterior spiracle in second and third instars has a single row of papillae varying in number from 14 to 17. The posterior spiracular discs have incomplete peritremes, with a prominent inner arc. Three long, narrow spiracular slits are oriented more or less vertically in each spiracular disc of third instar. Posterior spiracular hairs lack extensive branching and emanate approximately midway down the length of each slit. Microscopic morphology of the mouthhooks markedly differs between the first and second instars.

Sukontason *et al.* (2004a) differentiated the third instar of forensically important fly species in Thailand by using light microscopy, based on their morphological criteria for fly identification. Four species of the family Calliphoridae viz *C. rufifacies*, *C. megacephala*, *C. nigripes* Aubertin, 1932 and *L. cuprina* and two species of the family Muscidae viz *M. domestica* and *H. spinigera* Stein, 1910 were examined in this study based on features of the anterior spiracles, dorsal spines between the prothorax and mesothorax and posterior spiracles.

Sukontason *et al.* (2004b) also identified forensically important eggs of *C. rufifacies*, *C. megacephala*, *C. pacifica* Kurahashi, 1991, *C. nigripes*, *Aldrichina graham* Aldrich, 1930, *Lucilia cuprina*, *M. domestica* and *Megaselia scalaris* Loew, 1866 by using potassium permanganate staining technique. The characteristics are based on the width of plastron, morphology of plastron area surrounding the micropyle and chorionic sculpturing, with the length of egg being used as supplemental feature.

Triplehorn and Johnson (2005) described and provided a key for the families of Diptera.

Sukontason *et al.* (2005a) described the morphology of all instars of *C. nigripes* with the aid of both light microscopy and scanning electron microscopy (SEM). Morphological features of the cephalopharyngeal skeleton, anterior spiracle, posterior spiracle and dorsal spines between the prothorax and mesothorax are highlighted. No consistent features were found, even using SEM, for distinguishing the first instar of *C. nigripes* from that of *C. megacephala* or *C. rufifacies*, two other commonly associated

blow fly species in corpses in Thailand. Several features observed in second and third instars proved to be valuable characteristics for separating these species.

Sukontason *et al.* (2005b) also morphologically described second and third instars of *C. villeneuvei*. Both instars were of hairy appearance, bearing elongated tubercles along the abdominal and caudal segments. The anterior spiracle had 13–15 papillae. Minute dark spots were observed to thoroughly cover the tubercle's surface, with 4–6 strong dark tips. Regarding the third instar, the intersegmental spines between the prothorax and mesothorax were heavily pigmented. The posterior spiracle had a thick and heavily pigmented incomplete peritreme. The surface and tip of the tubercles was covered with heavily pigmented sharp spines. The integument of the body was covered with numerous distinct net-like patches.

Sukontason *et al.* (2007) described the sensilla of the antenna and palp of *Hydrotaea chalcogaster* by using scanning electron microscopy. The antennal scape had one type of sensillum, the sharp-tipped sensillum trichodeum; whereas, the antennal pedicel also possessed this type of sensillum in addition to an unidentified type. The arista is located dorso-laterally on the flagellum and had three segments.

Szpila and Pape (2008) described the morphology of the first instar of the housefly *M. domestica* by using scanning electron microscopy. The following morphological structures were documented: antenna, maxillary palpus, facial mask, spinulation, posterior spiracles, anal pad and integumental micropores.

Carvalho and Patiu (2008) presented a key to the adults of 12 families of diptera whose species are found on carrion, including human corpses. A summary for the most common families of forensic importance in South America, along with a key to the most common species of Calliphoridae, Muscidae and Fanniidae and to the genera of Sarcophagidae were provided.

Giroux and Wheeler (2009) described the systematics and phylogeny of the subgenus *Sarcophaga* (*Neobellieria*) (Diptera: Sarcophagidae).

Whitmore (2009) reviewed the *Sarcophaga* (Diptera: Sarcophagidae) of Sardinia and provided a key to males and females of Sardinian and Corsican species of *Sarcophaga*.

Brink (2009) provided key diagnostic descriptions for the immature stages of forensically important Calliphorids and Sarcophagids in central South Africa. A range of characteristics was evaluated by means of light and scanning electron microscopy and the most pertinent of these were identified for diagnostic purposes. All the species could be identified by means of five morphological characteristics (labrum, cephalopharyngeal skeleton, posterior spiracles, spiracular plate and anal area).

Pascal *et al.* (2010) conducted morphological studies using optical and electronic microscopy to establish consistent characters of larval stages of *C. vicina*, *Synthesiomya nudiseta* Van der Wulp, 1883 for quick identification and to determine whether the distinctive characters can be properly observed by optical microscopy, since this would be a faster way to yield timely results for forensic purposes.

Sukontason *et al.* (2010) studied the larval morphology and developmental rate of forensically important flesh fly species in Thailand viz *Sarcophaga dux*, *S. ruficornis* and *S. peregrina* Robineau-Desvoidy, 1830 which were the species of greatest forensic importance. They described the third instar and adult male characteristics to identify these species. They also described the keys for identification of third instar larva and males of *Sarcophaga*.

Whitworth (2010a) described keys to the blowfly species (Diptera Calliphoridae) of America, North of Mexico.

Thyssen (2010) described the anatomical features of the immature stages of insects and taxonomic keys utilized to identify order, family and species of Brazilian forensically relevant insects with emphasis on larvae of the major Dipteran families.

Velasquez *et al.* (2010) described the morphological characteristics that allow the accurate identification of third instars of the species present in the Iberian Peninsula in the form of a diagnostic key.

Mendonc *et al.* (2010) described ultramorphological characteristics of immature stages (egg, larva and pupa) of *Chrysomya albiceps* Wiedemann, 1819 which is the fly species important in forensic entomology by scanning electron microscopy with detailed information about morphological characters helping to identify the immature forms and consequently serve as a tool in crime scene investigations.

Szpila (2010) provided keys for the identification of third instar larva and adults of European blowflies (Diptera: Calliphoridae) of forensic importance.

Whitworth (2010b) generated keys to 11 genera and 21 species of Calliphoridae found in the West Indies and redescribed five little known species of *Lucilia* such as species *Lucilia fayeae* Whitworth, 2010 and redescribed *Calliphora maestrica* Gonzalez-Mora & Fernandez, 1998.

Vairo *et al.* (2011) developed a pictorial identification key for species of Sarcophagidae (Diptera) of potential forensic importance in southern Brazil. Photographs of the main structures used in species identification mainly from the male terminalia were provided.

Meiklejohn (2012) studied the taxonomy and systematics of Australian *Sarcophaga* (Diptera: Sarcophagidae). They provided updated key and LUCID key with a comprehensive database of illustrations and photographs of male terminalia as well as biological and distributional information for each species of *Sarcophaga*. A molecular based DNA barcoding of CO1 was evaluated for species level identification of Sarcophagids. They validated DNA barcoding as a suitable method for molecular identification of all life stages of Australian Sarcophagids.

Couri *et al.* (2012) described a key to the genera for diagnoses, new records and description of a new species of Muscidae from Namibia. The muscid fauna of Namibia is known to comprise 57 species in 19 genera, with *Musca* being the most species rich genus.

Szpila *et al.* (2013) described the morphology and identification of first instars of the European Luciliinae and Mediterranean blowflies of forensic importance and provided a key to the first instars of European species of *Lucilia*.

Sanit *et al.* (2013) studied morphology and identification of eggs of forensically important flies including *Chrysomya rufifacies*, *C. megacephala*, *C. pinguis* Walker, 1858, *C. nigripes*, *Hypopygiopsis tumrasvini* Kurahashi 1977, *Lucilia cuprina*, *L. porphyrina* Walker, 1856 and *M. domestica* by using 1% potassium permanganate solution for 1 min. The key for identifying these eggs together with other reported species in Thailand was updated.

Vikhrev (2013) revised key for males of the *Hydrotaea irritans* Fallén, 1823 species-group and discussed the difficulties of identification of females of the *H. irritans* group.

Irish *et al.* (2014) described the keys for adults of Afrotropical species of the genus *Chrysomya* (Diptera: Calliphoridae). Notes on the distribution and biology of the 12 species were given.

Williams and Villet (2014) described the morphological characters to identify *L. sericata* and *L. cuprina*. They described morphologically the hybrids of *Lucilia sericata* and *L. cuprina* for the first time.

Sukontason *et al.* (2014) reviewed the morphology, bionomics and medical involvement of *Sarcophaga dux*. Important morphological characteristics of egg, larva, puparia and adult were highlighted with illustration and/or micrographs. Search

pertaining to molecular analysis used for fly identification and developmental rate of larvae were included.

Akbarzadeh *et al.* (2015) prepared a high-quality key to the adults of all species of forensically relevant blowflies of the Middle East. They observed that the new key can be easily applied by investigators inexperienced in the taxonomy of blowflies.

Vikhrev (2015) reviewed the *Hydrotaea parva* and *H. glabricula* groups (Diptera: Muscidae) and also gave the identification key for *H. parva* Meade, 1889 and *H. glabricula* Fallen, 1825 group for the palaeartic and oriental regions.

Oliveira *et al.* (2015) used scanning electron microscope to analyze and document the external anatomy of both the larval and adult stages of the black soldier larva. Changes in each step of their life cycle were noted as the insect grew and matured.

2.3 Molecular identification-DNA barcoding

Molecular method of identification is faster and has increased sensitivity and specificity. One such molecular method is DNA barcoding and this approach was employed for confirmation of morphologically identified insects. Therefore the following literature has been reviewed with respect to DNA barcoding.

2.3.1 In India

Kumar *et al.* (2013) did DNA barcoding for identification of sand flies (Diptera: Psychodidae) in India. A total of 62 specimens belonging to seven morphologically identified species of two genera *Phlebotomus* and *Sergentomyia* collected from

Puducherry Union Territory, Maharashtra and Rajasthan states of India were subjected to the analysis. Neighbor-joining (NJ) analysis of DNA barcode sequences identified the individuals of seven morphological species into eight distinct species, as presented in the designed NJ tree. This methodology delineated morphologically identified species, *S. bailyi*, into two genetically isolated groups.

Archana *et al.* (2014) did DNA barcoding of commonly prevalent *Culicoides* midges in South India. The barcoding of Cytochrome oxidase I (COI) gene of *C. anophelis* Edwards in 1922, *C. palpifer* Gupta and Ghosh, 1956, *C. huffi* Causey, 1938, *C. innoxius* Sen and Gupta, 1959 and *C. circumscriptus* Kieffer, 1918 yielded an amplified fragment of 648 bp sequence. Barcode for all 5 species was generated using BoldSystems v3 and submitted to GenBank for accession numbers. DNA barcoding enabled exact identification of 5 prevalent species.

Ramaraj *et al.* (2014) reported the occurrence of dipteran fly, *Chrysomya megacephala* for the first time from Royapuram fishing harbour, Chennai, Tamil Nadu, South East India. Larvae, pupae and adults were identified by morphological features and molecular tools. Molecular identification through generation of DNA barcoding using mitochondrial COI gene of *C. megacephala* was detailed.

Sharma *et al.* (2015) did molecular identification of forensically important Indian species of flesh flies (Diptera: Sarcophagidae) by using COI gene of Mitochondrial DNA. They used 450 bp fragment of COI gene for differentiation of ten forensically significant species of flesh flies in India. The outcome displayed the robustness of COI gene as a

diagnostic marker, since its nucleotide variability endows reliable distinction to be drawn between species.

Bhaskaran and Sebastian (2015) did molecular barcoding of green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae) using COI gene sequences. Amplified and sequenced a 545 bp fragment of cytochrome oxidase subunit I (COI) gene of *L. sericata*. The consensus sequence was searched for its similarity using BLAST programme of NCBI. The phylogeny tree construction and sequence divergence study were done.

Banerjee *et al.* (2015) did DNA barcoding of Tabanid (Diptera) vectors of surra disease in India. They used barcode gap and reciprocal monophyly (neighbor-joining and Bayesian tree) criteria to analyze barcode data. A total of 46 specimens belonging to 7 species under four genera in two subfamilies were used for study. DNA barcode data was not available previously for these species. Analysis revealed that all morphologically identifiable species can be discriminated using DNA barcoding data.

2.3.2 In different parts of the world

Folmer *et al.* (1994) described universal DNA primers for PCR amplification of a 710-bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) from 11 invertebrate phyla: Echinodermata, Mollusca, Annelida, Pogonophora, Arthropoda, Nemertinea, Echiura, Sipuncula, Platyhelminthes, Tardigrada, and Coelenterata, as well as the putative phylum Vestimentifera. Preliminary comparisons revealed that these COI primers generated informative sequences for phylogenetic analyses at the species and higher taxonomic levels.

Hebert *et al.* (2003) established the mitochondrial gene cytochrome *c* oxidase I (COI) to serve as the core of a global bioidentification system for animals. COI identification system provided a reliable, cost-effective and accessible solution to the current problem of species identification. Its assembly could also generate important new insights into the diversification of life and the rules of molecular evolution.

Nelson (2008) did molecular identification of forensically important blowflies (Diptera: Calliphoridae: Chrysomya). In his study COI barcode was found to be successful in identifying *Chrysomya* species from East Coast of Australia.

Monko *et al.* (2009) studied morphology and mitochondrial DNA barcoding of the flesh fly *Sarcophaga argyrostomaan* Robineau-Desvoidy, 1830 an important species in forensic entomology. Nucleotide sequence differences between specimens varied between 0.6–2.6%. Sequence derived from three specimens in the study fulfilled the DNA barcoding standards for species identification.

Rivera and Currie (2009) tested the efficacy of a 615-bp fragment of the cytochrome *c* oxidase I (COI) as a DNA barcode in the medically important family Simuliidae. A total of 65 (25%) morphologically distinct species and sibling species in species complexes of the 255 recognized Nearctic black fly species were used to create a preliminary barcode profile for the family. They concluded that DNA barcoding was an effective method for species identification and discovery of cryptic diversity in black flies.

Paredes-Esquivel *et al.* (2009) standardized DNA barcoding techniques to identify *Culicoides* species in Europe. They standardized the methodology and the selection of the fragment to be amplified to homogenize results obtained by different groups.

Reibe *et al.*, (2009) did molecular identification of forensically important blowfly species (Diptera: Calliphoridae) from Germany. They checked a 229 bp sequence within the mitochondrial cytochrome oxidase subunit I. The sequences of the local flies were aligned to published data of specimens from other countries.

Hamada *et al.* (2010) did DNA barcoding for *Simulium litobranhium* n. sp. and three other closely related species in the Neotropical subgenus *S. (Thyrsopelma)* *Simulium litobranhium* n. sp. Mattos-Gloria & Luz, 2010 exhibited more than 4% nucleotide divergence in cytochrome oxidase I from three other closely related species in the Neotropical subgenus *S. (Thyrsopelma)*. The high divergence (> 4%) in the sequences of the COI gene among the four species suggested that they were different species.

Lin *et al.* (2010) studied population analysis of forensically important Calliphoridae on pig corpses in Taiwan. They provided the keys for identification of Calliphorids and also used COI sequencing for identification of flies. The COI sequences of six blowfly populations found in Taiwan were aligned and phylogenetic analysis was performed by using Neighbor-joining method. The result showed that phylogenetic relationships from molecular data highly agree with that from the morphological data.

Pramual *et al.* (2011) assessed the utility of DNA barcoding, based on cytochrome c oxidase subunit I (COI) sequences, for identifying 13 species of Oriental black flies in

the subgenus *Gomphostilbia*. Despite a low level of success, they found that DNA barcoding was useful in revealing cryptic biodiversity, potentially facilitating traditional taxonomy. Phylogenetic analyses indicated that species groups currently recognized on morphological criteria were not monophyletic, suggesting a need to re-evaluate the classification of the subgenus *Gomphostilbia*.

Kim *et al.* (2012) did DNA barcoding based on COI sequences from 156 species (529 sequences) of fish, insects, and shellfish. They presented results on phylogenetic relationships to assess biodiversity in the Korean peninsula. Their results confirmed that distance-based DNA barcoding provided sufficient information to identify and delineate fish, insect, and shellfish species by means of all possible pair wise comparisons and also confirmed that the development of an effective molecular barcode identification system was possible.

Puente *et al.* (2012) captured biting midges on two farms in the Canary Islands to identify the midge species in question and to characterize their COI barcoding region and to ascertain the source of their bloodmeals using molecular tools.

Ander *et al.* (2012) did barcoding of biting midges in the genus *Culicoides* a tool for species determination. The sequencing of mitochondrial cytochrome oxidase subunit I (COI), referred to as DNA barcoding, was proposed as a tool for rapid identification of species.

Boehm *et al.* (2012) used mitochondrial DNA cytochrome oxidase I gene barcoding region as a universal marker for molecular identification of forensically

important Diptera. They analyzed 111 specimens belonging to 13 species originating from Germany. (Calliphoridae and Muscidae). Although differences within species were generally less than among species, divergence percentages overlapped due to low interspecific nucleotide divergence. However, all species formed distinct monophyletic clades and thus the cytochrome oxidase 1 (COI) barcode has been shown suitable for clear differentiation and identification of forensically relevant Diptera in Germany.

Renaud *et al.* (2012) did DNA barcoding of Northern Nearctic Muscidae and generated a DNA barcode reference library for nearly half the northern Nearctic Muscidae and contributes almost all of the vouchered barcode records for this family now available through BOLD.

Xiong *et al.* (2012) used cytochrome oxidase subunit I (COI) for Identification of the forensically important flies (Diptera: Muscidae) in China. 31 forensically important Muscidae flies were collected from 15 locations in 11 provinces of China, and a 272 base pair region of the mitochondrial cytochrome oxidase subunit I (COI) gene was sequenced.

Aly and Wen (2013) assessed the applicability of partial characterization of cytochrome oxidase I in identification of forensically important flies (Diptera) from China and Egypt.

Gilarrortua *et al.* (2013) investigated the cytochrome b as a useful tool for the identification of blowflies of forensic interest (Diptera, Calliphoridae). Six species belonging three genera of Calliphoridae were collected and identified. The results showed

the robustness of the cyt b locus as a diagnostic tool, since its nucleotide variability enables reliable distinctions to be drawn between species. Their study contributed new consensus sequences which may be of interest for correct species identification.

Meiklejohn *et al.* (2013) did DNA barcoding to identify all immature life stages of a forensically important fleshfly (Diptera: Sarcophagidae). Amplification of the barcoding region was successful from all extracts, but puparia amplicons were weak. All sequences were identified as *Sarcophaga impatiens* Walker, 1849 with 99.95% confidence using the Barcoding of Life Database (BOLD). The results of their study indicated that immatures of *S. impatiens*, either larvae or puparia, are an adequate tissue source for molecular species-level identification using DNA barcodes.

Jordaens *et al.* (2013) identified forensically important *Sarcophaga* species (Diptera: Sarcophagidae) using the mitochondrial COI gene. Using two simple criteria (Best Match, BM and Best Close Match, BCM), they showed that the identification success using a mini-barcode region of 127 bp was very low (80.7-82.5 %) and the use of this region is not recommended as a species identifier. In contrast, identification success was very high using the standard barcode region (658 bp) or using the entire COI region (1,535 bp) (98.2-99.3 %).

Rolo *et al.* (2013) identified sarcosaprophagous Diptera species through DNA barcoding in wildlife forensics. During their study a portion of COI was sequenced from 95 specimens of seven species belonging to two families of Diptera (Calliphoridae and Muscidae) found in wildlife carcasses. All specimens were identified at species level with

a high specimen similarity and maximum identity percentage (through BOLD Systems and GenBank online databases, respectively).

Pramual *et al.* (2014) did DNA barcoding of tropical blackflies (Diptera: Simuliidae) of Thailand. A total of 351 cytochrome c oxidase subunit 1 sequences were obtained from 41 species in six subgenera of the genus *Simulium* in Thailand. Despite high intraspecific genetic divergence DNA barcodes provided 96% correct identification.

Aly (2014) investigated reliability of long vs short COI markers in identification of forensically important flies. They reported that in dipterous identification, it is recommended to use the long COI marker due to its greater reliability and safety.

Gutierrez *et al.* (2014) conducted DNA barcoding for the identification of sandfly species (Diptera, Psychodidae, Phlebotominae) in Colombia. They sequenced 700 bp of the COI gene from 36 species collected from different geographic localities. Their results indicated that the barcoding gene correctly discriminated among the previously morphologically identified species with an efficacy of nearly 100%.

Polseela *et al.* (2015) did DNA barcoding of sandflies of Wihan Cave, Uttaradit DNA barcodes generated for 193 sandflies fell into 13 distinct species clusters under four genera (*Chinius*, *Idiophlebotomus*, *Phlebotomus* and *Sergentomyia*). The species checklist and DNA barcode library contributed to a growing set of records for sandflies which is useful for monitoring and vector control.

Arnaldos *et al.* (2015) did COI barcoding sequence of two species of forensic interest, *Sarcophaga tibialis* Macquart, 1851 and *S.cultellata* Pandelle, 1896 from Spain.

A first analysis of 61 species of the genus *Sarcophaga* with all available information obtained from GenBank and BOLD databases, showed that success in correct identification.

Nzulu *et al.* (2015) did DNA barcoding for identification of sandfly species (Diptera: Psychodidae) from leishmaniasis-endemic areas of Peru. They generated DNA barcodes of the cytochrome oxidase subunit I (COI) gene using 159 adult specimens morphologically identified to be 19 species of sand flies, belonging to 6 subgenera/species groups circulating in Peru. Neighbor-joining (NJ) analysis based on Kimura 2-Parameter genetic distances formed non-overlapping clusters for all species. The generated COI barcodes could discriminate between all the sandfly taxa.

Pinto *et al.* (2015a) did DNA barcoding of neotropical sandflies (Diptera, Psychodidae, Phlebotominae) species identification and discovery within Brazil. DNA barcodes correctly identified approximately 90% of the sampled taxa (42 morphologically distinct species) using clustering based on neighbor-joining distance. They reported the effectiveness of DNA barcoding for species identification and the discovery of cryptic diversity in sand flies from Brazil.

2.4 Evaluation of new modes of application with semiochemicals

Chemical control of house fly facing many problems Therefore, identification of novel effective alternative strategies to manage this important pest by using more eco-friendly methods such as use of semiochemicals are of paramount importance for the development of successful control against the housefly. Therefore the following literature has been reviewed with respect to semiochemicals.

2.4.1 In India

Latha and Ranju (2011) in detail reviewed the different types of semiochemicals of ticks and new approaches of tick control by using semiochemicals in combination with acaricides to attract and kill ticks.

Latha (2012) reviewed the use of semiochemicals as a novel method for tick control by using semiochemicals in combination with acaricides to attract and kill ticks.

Ranju *et al.* (2012) tested the effect of attractant sex pheromone on immature larval stages of ixodid tick species. Attractant sex pheromone (ASP) 2, 6-dichloro phenol was evaluated for the percentage attraction and the behavioural responses of the five ixodid tick species namely *R. sanguineus* Latreille, 1806, *R. microplus* (*Boophilus microplus*) Canestrini, 1888, *Haemaphysalis bispinosa* Neumann, 1897, *R. haemaphysaloides* Supino, 1897 and *Hyalomma marginatum* Koch 1844 using petridish bioassay. Two concentrations of 2,6-DCP (0.1 M and 0.05 M) was used for the larval stages of all five ixodid tick species of which 0.1 M concentration was found to have maximum attraction. Trials with 0.1 M ASP revealed highest per cent of attraction in *R. sanguineus* larvae (71 %) followed by *H. bispinosa* (55 %) and *R. microplus* (55 %). With 0.1 M ASP *R. haemaphysaloides* and *H. marginatum* showed least attraction (39 % each). The larvae also exhibited behavioural responses such as feeding, probing, resting and questing posture.

Ranju *et al.* (2013) conducted field trials to attract questing stages of brown dog tick, *R. sanguineus* using tick pheromone–acaricide complex. Tick lure comprising of carbon dioxide as kairomone and 2, 6-dichlorophenol in combination with assembly

pheromone and deltamethrin on a thermocol platform was prepared and field trials were conducted in the kennels of Blue Cross Society of India, Velachery, Chennai. The tick lure attracted larval, nymphal and adult stages of *R. sanguineus* ticks in and around the kennels. Observations were made after 24 h of placing the tick lure. In simulated field out of the 50 ticks used for each distance trials, positive response was exhibited by a total number of 20 (40 %), 17 (34 %) and 14 (28 %) ticks from 0.5, 1 and 1.5 m, respectively. Field trials conducted revealed considerable attraction of larvae and nymph to the tick lure. Few adult ticks (2–5) were also lured to the pheromone impregnated filter paper disc.

Bino Sundar *et al.* (2014) studied the efficacy of the housefly specific pheromone (Z)-9-Tricosene in attracting and killing houseflies in field conditions using a sticky trap baited with sugar. Significant difference in the number of flies was observed in Z-9-Tricosene treated traps when compared to control traps without (Z)-9-Tricosene thus indicating the effectiveness of (Z)-9-Tricosene as an efficient lure in attracting house flies so that eco friendly pheromone based traps can be used in house fly control strategies as part of integrated pest management strategies.

Bhoopathy *et al.* (2014) conducted a novel approach to control brown dog tick, *R. sanguineus* using sustained release poly- ϵ -caprolactone-pheromone microspheres. The assembly pheromone, in defined ratio, was encapsulated using poly- ϵ -caprolactone by water-in-oil-in-water double emulsion solvent evaporation technique. In the in vitro bioassay, percent mortality with test microspheres was 95.6, 64 and 44 among the unfed larvae, unfed nymph and unfed adults respectively, 24 hours post-exposure. Field trials

were carried out to evaluate the efficacy of microspheres in luring and killing environmental stages of *R. sanguineus* in dog houses/kennels. Engorged and unfed stages in the environment were found adhered and dead on the specially designed lure.

Bhoopathy *et al.* (2014) evaluated the control of brown dog tick, *R. sanguineus* using assembly pheromone encapsulated in natural polymer, chitosan. Chitosan beads were prepared by incorporating assembly pheromone in conjunction with an acaricide, namely, deltamethrin. In the in vitro bioassay, the test beads attracted and killed 79 % of unfed larvae, 88 % of unfed nymphs and 61 % of unfed adults of the brown dog tick, *R. sanguineus*, in 24 h of exposure. Field trials were carried out to attract and kill the pre-parasitic environmental stages. The beads were dispersed onto specially designed devices and they were placed in infested kennels. The devices were observed after 10 days.

Bhoopathy *et al.* (2015) tested the response of unfed stages of *R. sanguineus* to subtle variations in the composition of assembly pheromone. Two combinations of assembly pheromone (AP), with and without hematin were utilized as a lure for the unfed larvae, nymph and adult *R. sanguineus* ticks. In-vitro trials were carried out with the AP encapsulated in calcium alginate beads and the response of different stages of ticks were recorded. Analysis of results revealed that rapid attraction was evident in unfed larvae exposed to beads containing AP without hematin. In case of unfed nymphal and adult stages, the presence or absence of hematin did not have any impact on arrestment.

Bino Sundar *et al.* (2016) evaluated (Z)-9-Tricosene based *M. domestica* lure study on a garbage dump yard using plywood sticky trap baited with fish meal. The traps were left in areas of the garbage dump yard of high fly activity for 6 h and then the

trapped flies were counted species wise. Significantly more number of *Musca domestica* flies were caught in (Z)-9-Tricosene treated fish meal baited traps compared to those traps without (Z)-9-Tricosene. In addition *Sarcophaga* sp. and *Chrysomyia* sp. flies were also caught in the traps.

Bhoopathy and Latha (2016) evaluated the ecofriendly approach to control brown dog tick, *R. sanguineus* using sustained release assembly pheromone beads. Sustained release calcium alginate test beads were prepared with synthetic assembly pheromone in conjunction with an acaricide. The response of unfed stages of brown dog tick to sustained release assembly pheromone test beads was studied by invitro and field trials. In vitro trails with test beads resulted in mortality of 100% of the exposed unfed larvae and adults and 90% of unfed nymphal stages ticks 24hrs post exposure. Field trials were conducted with specially designed lures. The lure was placed in kennels and was found to attract and kill the pre-parasitic environmental stages of *R. sanguineus*. This is a novel attempt to encapsulate assembly pheromone using biodegradable and environmental safe calcium alginate.

Ranju *et al.* (2016) tested the effect of attraction-aggregation-attachment pheromone and kairomone, carbon dioxide on adult tick of *R. sanguineus*. *Invitro* evaluations were made to observe the efficacy of attraction-aggregation-attachment pheromone (AAAP) and kairomone carbondioxide on particularly fed adult ticks (both male and female ticks) of *R. sanguineus* using olfactometer bioassay. Bioassays were conducted with 50 adult ticks each using three treatments namely AAAP in combination with carbon dioxide, carbon dioxide alone and AAAP alone. Dichloromethane (DCM),

diluents of AAAP served as control. Experimental trails using AAAP along with carbon dioxide resulted in 38% of *R. sanguineus* ticks showing attraction toward the pheromone source. AAAP alone attracted only 30% of ticks while carbon dioxide alone elicited an attraction of 76%. The effect of AAAP on partially fed adult ticks of *R. sanguineus* was found to be not substantial. However carbon dioxide showed a marked attraction among the partially fed adult male ticks of *R. sanguineus*.

2.4.2 In different parts of world

Chapman *et al.* (1998) evaluated three (Z)-9-Tricosene formulations for control of *M. domestica* in caged-layer poultry units. Targets baited with 5 g of technical grade (Z)-9-tricosene, or 5 g of a 40% polymer bead formulation, caught significantly greater numbers of *M. domestica* than control targets. This increase in attractiveness of the pheromone-impregnated targets persisted for at least 24 weeks. However, mean daily catch rates of *M. domestica* at targets baited with 5 g of a 2% wettable powder formulation did not significantly differ from control levels. Technical grade and bead formulations of the pheromone attracted significantly more males than females. However, the catches of female *M. domestica* at these pheromone-impregnated targets were significantly greater than female catches at control targets. Monitoring with sticky cards indicated that the introduction of toxic targets successfully suppressed adult *M. domestica* population density for up to 13 wk.

Chapman *et al.* (1999) evaluated the visual responses of *M. domestica* to pheromone impregnated targets in poultry units. Targets treated with azamethiphos and baited with 2.5 g of 40% (Z)-9-tricosene impregnated beads caught significantly greater

numbers of *M. domestica* than control targets. The greater attractiveness of the pheromone impregnated targets persisted for at least 5 weeks. The addition of longitudinal black stripes, or a regularly spaced pattern of black spots, to the white targets had no effect on catch rates. However, a pattern of clustered black spots, designed to imitate groups of feeding *M. domestica*, significantly increased target catches; this effect was particularly pronounced in the targets impregnated with (Z)-9-tricosene.

Hanley *et al.* (2004) evaluated the efficacy of (Z)-9-tricosene baited targets for control of the *M. domestica* in outdoor situations. Catch consisted largely of males, was consistently higher on larger traps, and generally increased with (Z)-9-tricosene concentration even up to very high levels. However, in repeated trials, and despite mass release of marked flies, catch rates appeared to be insufficient to provide adequate control. They suggested that this is probably because (Z)-9-tricosene is primarily a short-range attractant, and fly populations in outdoor situations are generally distributed over a large area. Catch declined rapidly to zero within 2 weeks, indicating that improved formulation or target design is needed to slow the weathering of active ingredients on the targets. Overall the trials suggest that (Z)-9-tricosene may be of little use in outdoor situations.

Pospischil *et al.* (2005) studied the development of new insecticidal compounds with oral action of baits for fly control. The attractiveness of the WG 10 formulation was increased by a mixture of z-9 tricosene and a new fly attractant (LEJ 179) developed by Bayer.

Logan and Birkett (2007) reported recent advances in chemical ecology, research of semiochemicals and their identification which showed great potential as control agents against biting insects.

Butler *et al.* (2007) evaluated the activity of houseflies near baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. Sticky card captures of *M. domestica* were used to compare efficacy of screen-covered baits containing sugar, sugar and 0.1% (Z)-9-tricosene, sugar and 1.0% (Z)-9-tricosene, Golden Malrin [1.1% methomyl and 0.049% (Z)-9-tricosene], and Quick-Bayt [0.5% imidacloprid and 0.1% (Z)-9-tricosene]. An average of 1.4, 5.6, 363.0, and 1,266.0 flies were killed using sugar, Golden Malrin, QuikStrike, and QuickBayt, respectively in 90min.

Nchu *et al.* (2009) conducted an experiment to explore the use of a semiochemical bait to enhance exposure of *Amblyomma variegatum* Fabricius, 1794 (Acari: Ixodidae) to different formulations of the entomopathogenic fungus *Metarhizium anisopliae* Sorokin, 1883. Simultaneous release of 1-octen-3-ol and AAAP together with CO₂ from a trap in semifield plots attracted up to $94.0 \pm 6\%$ of adult ticks from a distance of 6 m, and up to $24.0 \pm 5.1\%$ from 8 m. Formulations of *M. anisopliae* (dry powder, oil, and emulsifiable) applied within the trap baited with AAAP, 1-octen-3-ol and CO₂ resulted in high levels of contamination of the ticks attracted to the traps.

Butler and Mullens (2010) evaluated adult housefly activity near varying levels of (Z)-9-Tricosene on a Southern California dairy. Significantly more males than females were collected in the traps. Significantly more flies (male and female) were collected in

traps with (*Z*)-9-tricosene. There were no significant differences among doses of (*Z*)-9-tricosene alone, but numbers of both sexes were significantly higher in traps baited with (*Z*)-9-tricosene and sugar compared with the 5 μ l and 50 μ l doses without sugar.

Mann *et al.* (2010) evaluated nineteen semiochemicals classified as aliphatic alcohols, terpenoids, ketones and carboxylic esters and they showed toxicity to houseflies and stable flies.

Heuskin (2012) did a semiochemical slow-release formulation in a biological control approach to attract hoverflies. This research proved the efficiency of semiochemical alginate formulations as potential biological control tool to attract aphid predators.

2.5 Evaluation of biopesticides against fly control

Chemical control of house fly facing many problems. Therefore, identification of novel effective alternative strategies to manage this important pest by using more eco-friendly methods such as use of phytochemicals are of paramount importance for the development of successful control against the housefly. Therefore the following literature has been reviewed with respect to phytochemicals.

The relevant literature available for sweet basil (*Ocimum basilicum*), cumin seed (*Cuminum cyminum*), neem (*Azadirachta indica* A. Juss, 1830) and curcumin (*Curcuma longa*) were reviewed.

2.5.1 In India

Srinivasan and Amalraj (2003) tested the efficacy of insect parasitoid *Dirhinus himalayanus* Westwood, 1836 and insect growth regulator, triflumuron against housefly, *M. domestica*. The results showed that the combined use of parasitoid and insect growth regulator was effective in reducing puparia and fly density. Therefore, for sustenance of an effective fly control programme, both parasitoid and IGR may be used.

Murugan *et al.* (2007) investigated the larval toxicity and smoke repellent potential of *Albizzia amara* and *Ocimum basilicum* at different concentration (2%, 4%, 6%, 8% and 10%) against the different instar (I, II, III and IV) larvae and pupae of *Aedes aegypti*. The LC₅₀ values of *A. amara* and *O. basilicum* for I instar larvae was 5.412 and 3.734, II instar 6.480 and 4.154, III instar 7.106 and 4.664, IV instar 7.515 and 5.124, respectively. The LC₅₀ and LC₉₀ values of pupae were 6.792%, 5.449% and 16.925%, 15.474%. The smoke toxicity of *A. amara* was more active against *A. aegypti* than the *O. basilicum*.

Sumathi (2008) had observed that pupal parasitoids *Spalangia endius* and *Dihrinus* spp. inject their eggs with the help of ovipositor into the pupa of housefly. The newly hatched out larva were found to eat away the pupa and kill them and ultimately adult parasitoids emerged from the pupal case. A single parasitoid could infect 10 pupae (Cited by Ponnudurai 2013).

Ponnudurai and Harikrishnan (2008) evaluated the toxicity of *B. thuringiensis* to *M. domestica* which was related to Cry IAa (b), CryIB and Cry IIA protein. Under

laboratory condition, the spores (1 g in 50 g of larval medium) of *B. t. israeliensis* caused 50 % larval mortality.

Shanmugasundaram *et al.* (2008) evaluated the efficacy of neem (*Azadirachta indica*) and karanja (*Pongamia glabra*) oil cakes against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephens*. Both the oil cakes were applied individually and in combination. It was found that combination of neem and karanja oil cakes showed significant results as compared to individual treatments. The LC₉₅ for combination of two oil cakes was 0.93, 0.54 and 0.77% respectively for *C. quinquefasciatus*, *Ae. aegypti* and *An. stephens*.

Chaubey (2008) evaluated fumigant toxicity of seven spices among which *Cuminum cyminum* also evaluated against pulse beetle, *Callosobruchus chinensis* for its insecticidal, oviposition, egg hatching and developmental inhibitory activities. The essential oils caused death of adults and larvae when fumigated and also reduced the oviposition potential, egg hatching rate, pupal formation and emergence of adults from pupa when fumigated. They reported that toxic and inhibitory effects may be due to suffocation and inhibition of various biosynthetic processes of the insects at different developmental stages.

Aarthi and Murugan (2010) evaluated the larvicidal and repellent activity of *Ocimum basilicum* against the malarial vector, *Anopheles stephensi*. Laboratory investigation of *O. basilicum* showed 85% mortality.

Tamilam *et al.* (2010) tested the efficacy of thermocole coated with spores of *M. anisopliae* and *B. bassiana* against adult *M. domestica*. They tested at three concentrations (5×10^7 , 7.5×10^7 and 15×10^7 spores per ml) under laboratory conditions. All concentrations caused >90 per cent mortality of adult flies in 6-10 days in *M. anisopliae* whereas in case of *B. bassiana* 7.5×10^7 and 15×10^7 spores per ml produced >90 per cent mortality of flies.

Kannagi *et al.* (2010) isolated fungi from houseflies in poultry farms. Dead adult house flies collected from poultry manure in eight poultry farms revealed the presence of five species of fungi on their bodies. The isolated fungi were identified as *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., entomopathogenic fungi, *Metarhizium* sp., on houseflies is recorded for the first time in India.

Harikrishnan (2010) listed various biological control agents against houseflies such as several species of hymenopterous parasites particularly in the family Pteromalidae including *Spalangia endius* Walker, *Dirhinus himalayanus* and *Pachyrepoides vindemni* Rondani. The species of mites which prey on fly eggs or larvae belonged to *Macrochelidae*, *Uropodidae* and *Parasitidae*. The common Macrochelid mite was *Macrocheles muscae domestica* and the Uropid mite was *Fuscuraopoda vegetans*. Many beetles of Histeridae and Staphylinidae predated on egg and larvae. The major predaceous Histerid beetle was *Carcinops pumilio*.

Tamilam (2010) tested fungal baits containing *M. anisopliae* and *B. bassiana* under laboratory condition by smearing of castor oil impregnated with spores on thermocole and reported 90 per cent mortality in 6 days (Cited by Ponnudurai 2013).

Begum *et al.* (2010) evaluated toxicity potential and anti AChE activity of seeds of *Annona squamosa* and *Calotropis procera* in different developmental stages of *M. domestica*. The LC₅₀ values of the extracts of *C. procera* and *A. squamosa* seeds were found to be 870 and 345mg l⁻¹, respectively. The exposure of these extracts caused significant inhibition of the AChE activity in the developing phases of the insect. The high concentration (10%) of extract from the seeds of *A. squamosa* exhibited maximum inhibitory effects (56%) on the AChE activity from all the three developmental stages of the insect tested.

Mishra *et al.* (2011) tested the susceptibility of the adult and larval stage of housefly, *M. domestica* to two entomopathogenic fungi, *M. anisopliae* and *B. bassiana* under laboratory and simulated field bioassays. They reported better performance of *M. anisopliae* as an adulticidal and larvicidal agent over *B. bassiana* in laboratory bioassays as well as simulated field conditions suggesting that it may have good potential to become part of an integrated housefly control program.

Sumathi *et al.* (2011) tested the release of parasitoid wasps (Hymenoptera: Chalcididae and Pteromalidae) for control of houseflies in poultry farms. Mass release of laboratory reared *S. endius* and *D. himalayamus* simultaneously in adequate numbers at weekly intervals during the fly season may be an effective biological control measure for suppression of house fly population in small poultry farms.

Kannagi *et al.* (2011) evaluated the effect of *M. anisopliae* on the biological stages of *M. domestica*. Application of *M. anisopliae* to larvae, pupae and adults of the housefly at 1×10^6 , 1×10^7 and 1×10^8 conidia/ml resulted in mortality of the biological

stages (55-70%) and reduced house fly emergence (38.75%, 30% and 17.5%). *Metarhizium anisopliae* spores used in conjunction with soya oil exhibited the highest pathogenicity on *Musca domestica* larvae causing adult mortality rates of 85%. Dead flies from treated groups showed the presence of fungal mycelia on their bodies.

Harikrishnan (2011b) reported the preparation of entomopathogenic fungi incorporated baits for fly control.

Kamaraj *et al.* (2011) evaluated the larvicidal and repellent activity of *Acacia concinna*, *Cassia siamea*, *Coriandrum sativum*, *Cuminum cyminum*, *Lantana camara*, *Nelumbo nucifera*, *Phyllanthus amarus*, *Piper nigrum* and *Trachyspermum ammi* against *Anopheles stephensi* and *Culex quinquefasciatus*. *C. cyminum* which showed 100% mortality.

Kumar *et al.* (2012a) evaluated the insecticidal activity of essential oil of *Citrus sinensis* against larvae and pupae of the housefly. In the contact assay, LC_{50} of *C. sinensis* essential oil against housefly larvae, varied between 3.93 and 0.71 $\mu\text{l}/\text{cm}^2$ for different observation days, while lethal time, LT_{50} , varied between 5.8 to 2.3 days. In fumigant assay for housefly larvae, LC_{50} of 71.2 and 52.6 $\mu\text{l}/\text{l}$ was obtained in 24 and 48 h, respectively. Percentage inhibition rate of oil against housefly pupae was 27.3–72.7% for contact toxicity and 46.4–100% for fumigation assay.

Kumar *et al.* (2012b) evaluated eucalyptus globules for its chemical composition and insecticidal activity against the housefly, *M. domestica*. Insecticidal activity of *E. globulus* oil was assessed against larvae and pupae of housefly, through two different

bioassays: contact toxicity and fumigation. Contact toxicity assay with larva showed lethal concentration, LC_{50} , between 2.73 and 0.60 $\mu\text{l}/\text{cm}^2$ for different observation days while lethal time, LT_{50} , varied between 6.0 and 1.7 days. In fumigant assay for housefly larvae, LC_{50} values of 66.1 and 50.1 $\mu\text{l}/\text{l}$ were obtained in 24 h and 48 h, respectively. Pupicidal effectivity was measured in terms of percentage inhibition rate (PIR) which was 36.0–93.0% for contact toxicity and 67.9–100% for fumigation toxicity assay. Considerable activity of *E. globulus* oil against larvae and pupae of housefly demonstrated its potentiality as a viable option for the development of eco-friendly product for housefly control.

Kumar *et al.* (2012c) evaluated the efficacy of *Mentha* × *piperita* and *M. citrata* essential oils against *M. domestica*. Insecticidal activity of oils was assessed against larvae and pupae of housefly, through two different bioassays: contact toxicity and fumigation. For the larvicidal assay, lethal concentration, LC_{50} of *M.* × *piperita* oil, was 0.54 $\mu\text{l}/\text{cm}^2$ (contact toxicity) and 48.4 $\mu\text{l}/\text{L}$ (fumigation) while for *M. citrata* oil, it was 1.39 $\mu\text{l}/\text{cm}^2$ (contact toxicity) and 61.9 $\mu\text{l}/\text{L}$ (fumigation). Pupicidal effectivity was measured in terms of percentage inhibition rate (PIR). For, *M.* × *piperita* oil, PIR was 100% for both contact toxicity and fumigation toxicity assay while *M. citrata* oil showed PIR of 68% and 57% for contact toxicity and fumigation assay, respectively. The study established higher pupicidal and larvicidal efficacy of *M.* × *piperita* than *M. citrata* essential oil in contact toxicity as well as fumigation assay. The study demonstrated the potentiality of both the *Mentha* oils as a control agent against housefly with *M. piperita* being better candidate among the two.

Kumar *et al.* (2013) prepared and characterized *Mentha* × *piperita* oil emulsion for housefly control. Bioefficacy of emulsion against adults and larvae of housefly was found to be appreciable at various dilutions but showed slight decrease in efficacy with storage period.

Pawar (2013) studied the effect of *Curcuma longa* (Turmeric) on biochemical aspects of *M. domestica*. The biochemical aspects revealed that enzyme activities were decreased to 0.22 μmole/ml/min in case of Amylase while 0.30 μmole/ml/min decreased was observed in case of Invertase. It also decreased carbohydrate and protein content significantly i.e. 9.26 and 22.01 mg/gm body weight. Plant oil *C. longa* had the potential to be used in ecofriendly pest management. They concluded that this plant has to be further investigated for developing ecofriendly insect control pesticides.

Chintalchere *et al.* (2013) studied the bioefficacy of essential oils of Thyme (*Thymus vulgaris*) and Clove leaf (*Eugenia coryophyllus*) against housefly. The LC₅₀ 3.18 μg/cm² of clove leaf oil was found highly effective as compared to LC₅₀ 4.39 μg/cm² of Thyme essential oil for inducing mortality of *M. domestica* larvae. The adulticidal activity of Thyme essential oil LC₅₀ 32.71 mg/dm³ was toxic than clove leaf essential oil [LC₅₀ 53.10 mg/dm³]. In attractant / repellent bioassay, Thyme essential oil revealed 90.21% repellency as compared to 80.68 % value of Clove leaf essential oil against adults of the house fly. In fumigation bioassay, thyme showed high pupicidal activity than clove leaf oil and in contact toxicity bioassay using topical application both the oils showed 100 % pupicidal mortality. The data revealed that clove and thyme essential oils have excellent potential for controlling *M. domestica* population as eco-friendly approach in IPM.

Sumathi *et al.* (2013) did laboratory studies on *M. domestica* and its pupal parasitoid, *Dirhinus himalayanus* where they reported that *D. himalayanus* had the potential to be a biological agent of the house fly where caged layer poultry farms are maintained. *D. himalayanus* can be released in poultry farms at the parasitoid host ratio of 1:4.

Kumara *et al.* (2013) surveyed and isolated the different fungal pathogens against houseflies in different urban habitats. Only two fungal species viz., *B. bassiana* and *Aspergillus flavus* were isolated during the survey and there was no epizootic incidence of fungal pathogens during their study period.

Kumar *et al.* (2014) prepared and characterized PEG-Mentha oil nanoparticles for housefly control. Nanoparticles showed considerable mortality against housefly larvae in lab (100%) as well as simulated field condition after first week (93%) and 6th week (57%) of application. This was the first study utilizing controlled release property of nanoparticles to formulate a cost effective product for breeding site application against housefly.

Pandey *et al.* (2014) reviewed the chemical composition of essential oils of different *Ocimum* species. They reported a considerable difference in chemical composition of a particular species due to their occurrence in different eco-climatic zones and changes in edaphic factors and also reported various biological properties of *Ocimum* oils which are related to their various interesting applications as antimicrobial, antioxidant, repellent, insecticidal, larvicidal, nematocidal and therapeutic (anti-inflammatory, antinociceptive, antipyretic, antiulcer, analgesic, anthelmintic,

anticarcinogenic, skin permeation enhancer, immunomodulatory, cardio-protective, antilipidemic) agents.

Ali *et al.* (2014) reported the effect of *Curcuma longa* on *Tribolium castaneum* Herbst, 1797 a stored pest of wheat grain. It caused significant mortality and reduced the larval, pupal and adult emergence.

Rani and Harikrishnan (2016) tested invitro insecticidal activities of essential oils of *Eucalyptus globulus* against *M. domestica*. They extracted oil by hydrodistillation using Clevenger apparatus. In vitro efficacy of essential oil against larvae, pupae and adult flies at 0.2, 0.4, 0.6 and 0.8 percent concentration were evaluated. The LD₅₀ values of oil against larvae and pupae were 48.2 and 15.69µl respectively. The fumigant toxicity against adult flies showed that the highest concentration of 200 µl (0.8%) caused 100 percent mortality in 6.5±0.24 min.

Tamilam *et al.* (2016) tested the effect of *M. anisopliae* on reproduction of adult houseflies. In treated group, the average mean number of third stage larvae harvested in four trials was 69.2±12.3 as against 289.2±87.2 larvae from control group. In their study the mean number of third stage larvae harvested from *M. anisopliae* exposed group was lower than that of control group. This could be due to the fact that energy might have been diverted to fungal proliferation instead of egg development in fungi infected flies.

Rajesh *et al.* (2016) tested the biological effects of ethnoveterinary medicinal plant extracts on selected ectoparasites. The effect of various EVM plants biologically on selected ecto parasites. Plant viz. *Azadirachta indica*, *Syzygium cumini*, *Chloroxylon*

swietenia, *Treminalia chebula*, *Rubia cordifolia*, *Vitex altissima*, *Tinospora cordifolia*, *Curcuma amada*, *Acorus calamus* and *Trachyspermum ammi* were collected from natural forests of Namakkal districts, Tamil Nadu. The plants were sequentially extracted with five solvents such as hexane, chloroform, ethyl acetate, ethanol and water and tested against the larvae of *M. domestica*, *R. microplus* and *Culex* spp., at 1% concentration. The malformation and mortality of larvae were calculated and the treated larvae were maintained in laboratory for observation. In this study the larvicidal effects of medicinal plants revealed that the extracts of *R. cordifolia* and *T. chebula* found to have higher activity and *T. cordifolia* shows lower activity against *M. domestica*. *C. amada* has the highest activity and *V. altissima* has the lowest activity against *R. microplus* where as *A. indica* and *S. cumini* showed maximum activity and *V. altissima* showed minimum activity against the larvae of the *Culex* spp.

2.5.2 In different parts of world

West (1951) reviewed the history, medical importance, taxonomy, geographical distribution and control of house flies.

Rutz and Axtell (1979) investigated sustained releases of pupal parasite *Muscidifurax raptor* Girault & Sanders, 1910 (Hymenoptera: Pteromalidae) for housefly (*M. domestica*) control in two types (one with narrow houses and one with a high-rise house) of caged-layer poultry houses. Reduction in the fly population occurred in the narrow caged-layer houses; however, no reduction was evident in the high-rise caged-layer house.

Nolan and Kissam (1985) used *Ophyra aenescens* as a potential bio-control alternative for house fly' control in poultry houses. They reported that because of the predatory habits of the larvae, *O. aenescens* may be useful as a biological control agent as part of integrated pest management for house fly control on poultry farms.

Geden (1990) reported the natural predators of house flies such as beetle *Carcinops pumilio* Erichson and mite *Macrocheles muscae domesticae* Scopoli, 1772 feed on the house fly eggs and first stage larvae.

Barson *et al.* (1994) evaluated the effectiveness of six species of entomopathogenic fungi for the control of adult and larval stages of the house fly *Musca domestica* in the laboratory. *Metarhizium anisopliae* Sorokin, 1883 and *Tolypocladium cylindrosporum* W. Gams, 1971 were found to be most virulent to house fly larvae. Linseed oil was the most effective carrier of *M. anisopliae*, giving 100% kill in 3 days at a dose of 1×10^5 and in 6 days at 1×10^3 conidia/fly.

Geden *et al.* (1995) evaluated the virulence of different isolates and formulations of *Beauveria bassiana* Vuill, 1912 for houseflies and parasitoid *Muscidifurax raptor*. Adult flies were susceptible (94-100% mortality) to *B. bassiana* conidia of Hf88 isolate when applied to plywood boards, flies were also susceptible when confined with treated water or food. House fly larvae not affected in the rearing medium. The isolates were slightly more virulent for flies than for fly parasitoid *M. raptor*.

Khan and Ahmed (1998) evaluated the toxicity of crude neem leaf extract against housefly *M. domestica* adults. Neem leaf extract was applied at 5, 10, 20, 40 and 80

microgram/fly. LD₅₀ and LD₉₀ values were found to be 8.4 and 169.8 ug/fly of neem extract respectively. They stated that neem products may be used as population controlling agents for *M. domestica* as they are cheaper and biodegradable.

Weinzierl and Jones (1998) released *Spalangia nigroaenea* Curtis, 1839 and *M. zaraptor* to increase rates of parasitism and total mortality of stable fly and house fly pupae in Illinois cattle feedlots. Over the 3-yr period, releasing these species, particularly *S. nigroaenea*, significantly reduced production of stable fly and house fly adults in cattle feedlots. The potential value of such reductions is likely to vary as a result of feedlot conditions and weather.

Carswell *et al.* (1998) tested the susceptibility of adult houseflies, *M. domestica* and *Bactrocera tryoni* Froggatt, 1897 to an isolate of *Metarhizium anisopliae* at 20, 25 and 30°C. Both species of fly started to die from the disease after 4-5 days incubation at 25°C and 30°C and 100% mortality was reached at these temperatures after 7-9 days. They concluded that *M. anisopliae* may be suitable for control of both species in situations where chemical pesticides cannot be used.

Wimalaratne *et al.* (1999) isolated and identified the repellents from the pepper tree against the housefly. The volatile extracts of pepper tree leaves were shown to have repellent and feeding deterrent activity against houseflies in a two-choice laboratory bioassay, demonstrated that bioactivity is associated with two compounds, cis-menth-2-en-1-ol and trans-piperitol. Bioassays with house flies indicated trans-piperitol to be the most active house fly repellent.

Renn *et al.* (1999) observed that the spores of entomopathogenic fungi *M. anisopliae* and *B. bassiana* were highly pathogenic to adult flies and they are being used as biopesticide in temperate countries (Cited by Ponnudurai 2013).

Watson *et al.* (2001) tested the impact of the darkling beetle *Alphitobius diaperinus* a pest of chicken and turkey on establishment of the predaceous beetle *Carcinops pumilio* for *M. domestica* control in caged-layer poultry houses. Laboratory studies confirmed that larval darkling beetles significantly reduced the survival of *C. pumilio* eggs and larvae. Adult darkling beetles did not reduce *C. pumilio* egg or larval survival.

Renn *et al.* (2001) evaluated bait formulated with *M. anisopliae* for the control of *M. domestica* assessed in large-scale laboratory enclosures. After 8 days, 97.3% of female flies and 100% of male houseflies had succumbed to infection with *M. anisopliae*. Highly pathogenic to adult flies and they are being used as biopesticide in temperate countries.

Araujo and Leon (2001) reported biological activities of *Curcuma longa* anti-inflammatory, anti-human immunodeficiency virus, anti-bacteria, antioxidant effects and nematocidal activities.

Sukontason *et al.* (2004c) reported effect of eucalyptol on housefly and blowfly. The bioassay of adults, using topical application, indicated that *M. domestica* males were more susceptible than females, with the LD₅₀ being 118 and 177 µg/fly, respectively. A higher LD₅₀ of *C. megacephala* was obtained; 197 µg/fly for males and 221 µg/fly for

females. Living flies of both species yielded a shorter life span after being treated with eucalyptol. The bioassay of larvae, using the dipping method on the third instar, showed that *M. domestica* was more susceptible than *C. megacephala*, with their LC₅₀ being 101 and 642 µg/µl, respectively. The emergence of adults, which had been treated with eucalyptol in larvae, decreased only in *M. domestica*.

Kaufman *et al.* (2005) evaluated a commercially produced *Beauveria bassiana* product, balance and compared with pyrethrin treatments for the control of adult houseflies in New York high-rise, caged-layer poultry facilities. Adult housefly populations were lower in *B. bassiana*-treated facilities during the spray and post-spray periods, as recorded on spot cards. Concurrently, the numbers of house fly larvae recovered in *B. bassiana*-treated facilities were less than one-half that of the pyrethrin-treated facilities. The numbers of adult and larval *Carcinops pumilio*, a predatory beetle, recovered from *B. bassiana*-treated facilities were 43 and 66% greater than from the pyrethrin-treated facilities, respectively.

Popovic *et al.* (2006) evaluated biological activities of essential oils from *Ocimum basilicum* and *Salvia officinalis* against *Sitophilus oryzae* Linnaeus, 1763 in lab. Contact toxicity (applied essential oils on filter papers), grain treatment for mortality and repellency assays, and also effect of oils on insect fecundity were tested. Only most concentrated solutions of essential oils (2 %) of both plants caused the significant mortality, repellency and anti-reproductive effect. They suggested that these two plants can be incorporated into grain protection practices.

Tawatsin *et al.* (2006) evaluated the efficacy of 18 plant essential oils against *Aedes aegypti* Linnaeus in Hasselquist, 1762, *Ae. albopictus* Skuse, 1894, *Anopheles dirus* Peyton and Harrison, 1979 and *Culex quinquefasciatus* Say, 1823 under laboratory conditions using human volunteers was tested. For this purpose *Eleutherococcus trifoliatus*, *Schefflera leucantha*, *Ocimum sanctum*, *Vitex trifolia*, *Litsea cubeba*, *Manglietia garrettii*, *Aglaia odorata*, *Myristica fragrans*, *Melaleuca cajuputi*, *Psidium guajava*, *Piper betle*, *Piper nigrum*, *Murraya paniculata*, *Houttuynia cordata*, *Zingiber officinale*, *Alpinia galangal*, *Curcuma longa* and *Hedychium coronarium* essential oils were prepared with 10% absolute ethanol. These essential oils were compared with chemical repellents, DEET and IR3535. It was found that repellency of *An. dirus* Cx. *quinquefasciatus* and *Ae. albopictus* was 4.5-8hrs and repellency of *Ae. aegypti* was 0.3-2.8 hrs. It was found that oviposition deterrent activity of *C. longa* against *Ae. aegypti* was 94.7%, whereas DEET and IR3535 had no repellency. The results revealed that *C. longa* essential oil was an efficient mosquito repellent and oviposition deterrent.

Samarasekera and Kalhari (2006) evaluated insecticidal activity of essential oils of ceylon *Cinnamomum* and *Cymbopogon* species and their 10 compounds against *M. domestica*. *Cinnamomum zeylanicum* bark and *Cymbopogon citratus* oils showed good knock down and mortality against *M. domestica* (LD50 1.37 and 1.71 µg/insect, respectively) than *C. zeylanicum* leaf (LD50 2.75 µg/insect) and *C. nardus* (LD50 3.10 µg/insect) oils. Cinnamaldehyde, eugenol, eugenyl acetate, geraniol and geranyl acetate were more active against *M. domestica* than their corresponding essential oils, while cinnamyl acetate, citral, citronellal, citronellol and α -terpineol were less active. This study indicated that both the bark and leaf oils of *C. zeylanicum* and the leaf oils of

C. nardus and *C. citrates* grown in Sri Lanka could be used as potential protective agents against *M. domestica*.

Pavela (2007) evaluated lethal and sublethal effects of Thyme Oil (*Thymus vulgaris*) on the larvae and adults of housefly. The females, larvae and the male flies were more sensitive in the contact application. The application of sublethal doses to the adults of the housefly significantly decreased the longevity of both sexes. It was determined that the natural mortality of 50% of the treated adults occurred on the 5th - 7th day, to the contrary of 18th -19th day on which occurred the natural mortality of the non-treated adults. Thereby, the most significant mortality (more than 80%) was seen with larvae which emerged from eggs which were laid by the treated female flies. These larvae were non-vital, their pupae were significantly smaller and the survived pupae had a much higher mortality.

Okumu *et al.* (2007) tested larvicidal effects of a neem (*Azadirachta indica*) oil formulation (azadirachtin content of 0.03% w/v) on the malaria vector *Anopheles gambiae* Giles, 1902. Neem oil had an LC₅₀ value of 11 ppm after 8 days, which was nearly five times more toxic than the corn oil formulation. Adult emergence was inhibited by 50% at a concentration of 6 ppm. Significant reduction on growth indices and pupation, besides prolonged larval periods were observed at neem oil concentrations above 8 ppm. The corn oil formulation, in contrast, produced no growth disruption within the tested range of concentrations. Neem oil has good larvicidal properties for *A. gambiae* and suppresses successful adult emergence at very low concentrations.

Ruiu *et al.* (2007) tested the toxicity of a *Brevibacillus laterosporus* strain lacking parasporal crystals against *M. domestica* and *Aedes aegypti*. Major proteins, with a molecular weight of about 14 kDa, contained in fractions, are probably responsible for the observed toxicity. LC₅₀ values were 63.77 and 12.81 lg/g of diet for housefly larvae and adults, respectively, and 3.14 lg/ml for *A. aegypti* larvae. They suggested that the pathogenic activity of this *B. laterosporus* strain for *M. domestica* and *A. aegypti* is a toxin-mediated process reminiscent of the mechanism of action of *B. thuringiensis* d-endotoxins.

Dry *et al.* (2007) surveyed parasitoids (Hymenoptera: Pteromalidae) of house fly pupae from broiler-breeder poultry houses in northwest Arkansas. Five species of Pteromalidae were collected over a two-year sampling period: *Pachycrepoideus vindemiae*, *Spalangia endius*, *S. cameroni*, *Muscidifurax* spp., and *Nasonia vitripennis*.

McKay *et al.* (2007) sustained mass released pupal parasitoids *Muscidifurax zaraptor*, *M. raptorellus* and *Spalangia cameroni* for control of *H. aenescens* and *M. domestica* in broiler-breeder poultry houses in Arkansas. Sustained releases of parasitoids at the released farm over two years provided a significant increase in house fly pupal parasitism when compared to the percentage of pupae parasitized at the non-release farm. Also the sustained released of parasitoids reduced *H. aenescens* numbers to below the treatment threshold.

Birkemoe *et al.* (2009) tested the effect of biweekly mass release of *Spalangia cameroni* parasitoid of house flies and stable flies in southern Norway, on pig premises with scattered fly breeding sites. They reported that *S. cameroni* reduced densities of

house and stable flies on pig farms with scattered fly breeding sites. However, the effect depended on temperature.

Bisseleua *et al.* (2008) investigated the toxicity, growth regulatory and repellent activities of *Griffonia simplicifolia* and *Zanthoxylum xanthoxyloides* extracts on *M. domestica*. Seed extracts of *G. simplicifolia* and root extracts of *Z. xanthoxyloides* were the most effective as toxicants and repellents against the fly. The LD₅₀ in 24 h topical application of seed extracts of *G. simplicifolia* and root extracts of *Z. xanthoxyloides* were 0.28 and 0.35 µg, respectively.

Pavela (2008a) evaluated the insecticidal property of 34 essential oils against the housefly, *M. domestica*. Essential oils from *Pogostemon cablin* proved to be the most efficient at a lethal dose of 3µg/fly after topical application. Eight oils (*P. roseum*, *O. vulgare*, *O. compactum*, *M. pulegium*, *O. basilicum*, *O. majorana*, *T. vulgaris* and *P. graveolens*) were lethal in doses ranging from 10 to 20 µg (10, 13, 13, 13, 15, 17, 18 and 19 µg/fly, respectively). The lethal doses of another 13 oils were ascertained in the range 20–50 µg/fly, nine oils had lethal doses of 50–100 µg. In the fumigant test, the most efficient proved to be *Mentha pulegium* oil (4.7 µg/cm²). For 10 oils, a lethal dose between 5 and 10 µg/cm² was ascertained (*T. bipinata*, *C. aurantifolia*, *T. occidentalis*, *T. matschiana*, *S. officinalis*, *T. vulgaris*, *M. quinquenervia*, *O. compactum*, *C. limonum* and *R. officinalis*, respectively). For *O. basilicum* 53.5 µg/cm² the other 10 oils a lethal dose from 10 but to 80 µg/cm² was ascertained, and for 13 oils the lethal dose was higher than the highest dose in the tests.

Fabbro and Nazzi (2008) tested the repellency of sweet basil (*Ocimum basilicum*) against the tick *Ixodes ricinus* by using a laboratory bioassay. According to this study, eugenol from *O. basilicum* appeared to be as repellent as DEET at 2 tested doses.

Pavela (2008b) studied acute and synergistic effects of six monoterpenoid essential oil compounds on the house fly. Of the essential oils compounds tested for topical acute toxicity, the most potent were thymol and carvacrol, with LD₅₀ values < 100 µg per adult. In comparison, for fumigant acute toxicity the most potent were g-terpinene, p-cymene and cineole, with LD₅₀ values < 100 µg per cm³. Twelve binary mixtures out of 15 tested couples showed synergistic effects in topical application. The most significant synergism was found with p-cymene in combination with 1,8-cineole, g-terpinene or carvacrol. In the fumigant application, 8 binary mixtures showed a synergistic effect. The most profound synergistic effect was found in p-cymene with g-terpinene or with 1,8-cineole and g-terpinene in the mixture with 1,8-cineole.

Siriwattananurungsee *et al.* (2008) investigated the effect of neem, *Azadirachta indica*, on ultrastructural alteration of larvae and puparia of the *C. megacephala* and *M. domestica* in the laboratory by using neem extract containing 0.24% azadirachtin. Larvae of both species exposed to single dipping with the neem product exhibited swelling of the integument in relation to the control as determined by scanning electron microscopy (SEM). Transmission electron microscopy (TEM) analysis revealed slight thickness of epicuticle, but intense thickness of procuticle. Multiple treatment of the larvae displayed noticeably swelling integument and bleb formation on the integument, indicating a dose-dependent relationship. Puparia of both fly species treated with neem product showed similar appearance under SEM and TEM analyses.

Deleito *et al.* (2008) evaluated neem (*A. indica*) for controlling flies associated with animal production viz. *L. cuprina*, *C. megacephala*, *C. hominivorax* and *M. domestica*. Reduction of fly emergence from treated pupae with 0.6 per cent of neem oil was 95.6 per cent under laboratory conditions and 94.5 per cent when applied on the soil, where the cattle rest during the night. The application of neem oil at 0.6 per cent on the soil was a viable alternative for controlling pupae of *L. cuprina*, *C. megacephala*, *C. hominivorax* and *M. domestica*.

Mee *et al.* (2009) evaluated efficacy of *Piper aduncum* extract under laboratory conditions for its adulticidal activity against the *M. domestica* WHO 213 strain and the wild strain from Chow Kit, Kuala Lumpur. There were significant differences between the effects of *P. aduncum* extract on WHO 213 and wild Chow Kit ($p < 0.05$). *P. aduncum* extract, although less effective than the commercial product Resigen®. However, it is abundant in the tropics and could be utilized for adult housefly control.

Palacios *et al.* (2009a) evaluated insecticidal activity of nine essential oils (EOs) against the *Musca domestica*. They evaluated by placing flies in a screw-cap glass jar holding a piece of EO-treated cotton yarn. The LC50 in 30 min was determined where EOs showed LC50 values ranging from 0.5 to 46.9 mg/dm³. The EO from *Minthostachys verticillata* was the most potent insecticide (LC50=0.5 mg/dm³) followed by EOs from *Hedeoma multiflora* (LC50=1.3 mg/dm³) and *Artemisia annua* (LC50=6.5 mg/dm³). EOs from *M. verticillata* and *H. multiflora* showed promise as natural insecticides against houseflies.

Dua *et al.* (2009) evaluated plant essential oils of neem (*A. indica*), basil (*Ocimum basilicum*, *O. basilicum*, *O. citratum*, *O. gratissimum*, *O. americanum*, *O. tenuiflorum*, citronella grass (*Cymbopogon nardus*), galingale (*Alpinia galanga* L.), clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.), as a mosquito repellent. The efficacy of neem oil formulation against 3rd and 4th larval instars of *A. stephensi*, *Culex quinquefasciatus* and *A. aegypti* was tested. It was found that LC₅₀ against *A. stephensi*, *C. quinquefasciatus* and *A. aegypti* was 1.6, 1.8 and 1.7 ppm, respectively. LC₅₀ of formulation stored at 26°C, 40°C and 45°C for 48 hours against *Ae. aegypti* were 1.7, 1.7, 1.8 ppm while LC₉₀ values were 3.7, 3.7 and 3.8 ppm, respectively. The neem formulation at 140 mg A.I. /m² was applied in different mosquito breeding sites under field conditions. It was found that the maximum reduction rate of *Anopheles*, *Culex* and *Aedes* was 100%, 95.5% and 100%, respectively. The results revealed that neem oil formulation was found to be very effective to control *Anopheles*, *Culex* and *Aedes* species in different breeding sites under natural and field conditions. *A. indica* was as an alternative to synthetic pesticides for mosquito control.

Palacios *et al.* (2009b) tested efficacy of 12 essential oils from edible plants as insecticides against *M. domestica*. The LC₅₀ values ranging from 3.9 to 85.2 mg/dm³ was observed. *Citrus sinensis* was the most potent insecticide (LC₅₀ = 3.9 mg/dm³), followed by Eos from *C. aurantium* (LC₅₀ = 4.8 mg/dm³) and *Eucalyptus cinerea* (LC₅₀ = 5.5 mg/dm³). EOs from *C. sinensis*, *C. aurantium*, and *E. cinerea* showed promise as natural insecticides against houseflies.

Chang *et al.* (2009) reported that trans-anethole, estragol, and linalool are major bioactive compounds in basil oil against tephritid fruit flies. Methyl eugenol is a minor

component in basil oil, but it is very potent to *C. capitata* and *B. cucurbitae*. The action of these chemicals is fast and shows a steep dose-response relationship. If trans-anethole, estragole, linalool, methyl eugenol and basil oil are mixed with attractants such as paraphermones in an appropriate formula, they may be used as a natural insecticide.

Alouani *et al.* (2009) evaluated larvicidal activity of a neem tree extract (azadirachtin) against mosquito larvae in the Republic of Algeria. Mosquito adult fecundity was markedly decreased and sterility was increased by the Azadirachtin after treatment of the fourth instar and pupal stage. The treatment also prolonged the duration of the larval stage. The results showed that Azadirachtin was promising as a larvicidal agent against *Culex pipiens*.

Khater *et al.* (2009) evaluated lousicidal, ovicidal and repellent efficacy of camphor (*Cinnamomum camphora*), onion (*Allium cepa*), peppermint (*Mentha piperita*), chamomile (*Matricaria chamomilla*) and rosemary oils (*Rosmarinus officinalis*) and essential oils against lice and flies infesting water buffaloes in Egypt. The oils and d-phenothrin significantly repelled flies, *Musca domestica*, *Stomoxys calcitrans*, *Haematobia irritans* and *Hippobosca equine* Linnaeus, 1758, for 6 and 3 days post-treatment, respectively.

Kerdchoechuen *et al.* (2010) evaluated six herbal plants for biocontrol of the maize weevil among which sweet basil also evaluated. Sweet basil oil induced a relatively high mortality rate (greater than 90%) at a low volume of 30 mL against the maize weevil on 40 g of rice grain the first day of exposure and also completely suppressed insect progeny production thereafter.

Urzua *et al.* (2010) investigated the insecticidal properties of the essential oils from *Haplopappus foliosus* and *Bahia ambrosoides* against *M. domestica*. The essential oil from *Haplopappus foliosus* was the most potent insecticide ($LC_{50} = 4.43 \text{ mg/dm}^3$), while the EO from *Bahia ambrosoides* showed only moderate insecticidal activity ($LC_{50} = 19.27 \text{ mg/dm}^3$).

Nerio *et al.* (2010) reviewed repellent activity of essential oils. Among the plant families with promising essential oils used as repellents, *Cymbopogon spp.*, *Ocimum spp.* and *Eucalyptus spp.* were the most cited. Individual compounds present in these mixtures with high repellent activity include α -pinene, limonene, citronellol, citronellal, camphor and thymol.

Magdas *et al.* (2010) evaluated the acaricidal effect of eleven essential oils viz. Sweet basil, Common juniper, Atlas cedar, Coriander, Blue gum, European silver fir, Common lavender, Lemon, Peppermint, Scots pine and Summer savory, against the poultry red mite *Dermanyssus gallinae* De Geer, 1778 in vitro using the direct contact method, at three different doses: 0.2 mg/cm^2 , 0.4 mg/cm^2 and 0.6 mg/cm^2 . The results of the study revealed that oils of sweet basil, coriander, peppermint and Summer savory were the most effective.

Qing *et al.* (2010) evaluated anti-insect activity of the methanol extracts of fern and gymnosperm on both adult *M. domestica* and *Aedes albopictus*. Significant insecticidal activities were exhibited in the methanol extracts of five species, *Cupressus funebris* (leaves and stems), *Cycas acuminatissima* (roots), *Keteleeria fortunei* (leaves and stems), *Onychium japonicum* (whole plant), and *Pinus taiwanensis* var. *Daming*

shanensis (leaves and stems). Applications of the extracts of these plants resulted in higher than 50% mortalities in 4th instar larvae of *A. albopictus* at 24 h after treatment. The methanol extracts from 13 species possessed insecticidal activities against the adult of *M. domestica* at 48 h after treatment with higher than 90% mortalities. The extensive screening results showed that fern and gymnosperm were highly potential to be botanical insecticides.

Sharififard *et al.* (2011) investigated the interactions between entomopathogenic fungus, *M. anisopliae* and sublethal doses of Spinosad for control of housefly, *M. domestica*. The interaction between *M. anisopliae* and spinosad indicated a synergetic effect that increased the house fly mortality as well as reduced the lethal time.

Velazquez *et al.* (2011) evaluated acaricidal effect and chemical composition of essential oils extracted from *Cuminum cyminum*, *Pimenta dioica* and *Ocimum basilicum* against the cattle tick *Rhipicephalus (Boophilus) microplus* larvae by using the larval pocket test. Two-fold dilutions of the three essential oils were tested from a starting dilution of 20% down to 1.25%. Results showed a high toxicological effect for cumin, producing 100% mortality in all tested concentrations on *R. microplus* larvae. Similarly, all spice essential oil produced 100% mortality at all concentrations with the exception of a dramatic decrease at 1.25% concentration. Conversely, basil essential oil was not shown to be toxic against *R. microplus* larvae.

Ojianwuna *et al.* (2011) evaluated the toxicity of *Ocimum suave* (wild basil) leaf oil on adult houseflies. The oil applied at 0.05, 0.10, 0.15 and 0.20 mL/L caused

significant mortality of the housefly. The LC_{50} was 0.09 mL/50 mL of water, and the LT_{50} was 4.40 h.

Cisak *et al.* (2012) reviewed repellents and acaricides as personal protection measures in the prevention of tick-borne diseases. They observed that products based on natural compounds, e.g. eugenol from *Ocimum basilicum*, 2-undecanone originally derived from wild tomato, geraniol – a natural product extracted from plants and many others, represent an interesting alternative to common synthetic repellents and/or acaricides.

Mansour *et al.* (2012) evaluated the adulticidal activity of 12 plant species *A. indica*, *Cichorium intybus*, *Citrus aurantifolia*, *Conyza aegyptiaca*, *Eucalyptus globulus* (fruits and leaves), *Opuntia vulgaris*, *Piper nigrum*, *Punica granatum*, *Saccharum* sp., *Salix safsaf*, *Sonchus oleraceus*, and *Zea mays*. *P. nigrum* showed the highest toxicity ($LD_{50} = 0.115$ ug/insect), while *P. granatum* induced the lowest toxicity ($LD_{50} = 0.278$ ug/insect). Toxicity values of the other tested extracts ranged between the above mentioned values. *A.indica* showed 100% mortality at 1000 ppm with LD_{50} of 0.128 ug/insect. The study revealed the broad-spectrum toxic properties of the tested botanical extracts against the adult stage of *M. domestica*

Kiplangat and Mwangi (2013) evaluated repellent activities of *O. basilicum*, *A. indica* and *E. citriodora* extracts on rabbit skin against *A. aegypti*. Pyrethrum oleoresin showed complete protection at 0.1% as compared to Lemon Eucalyptus oil and Sweet Basil oil at 2% and 3% respectively ($p < 0.05$). Neem oil did not provide complete protection. The mean percent repellency of 5% Neem oil was 84.21.

Tavares *et al.* (2013) reported the effect of α -turmerone from *C. longa* (Zingiberaceae) rhizomes and effects on Maize weevil *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) and fall armyworm *Spodoptera frugiperda* J. E. Smith, 1797. The α -turmerone was highly toxic to *S. zeamais* and *S. frugiperda* at low doses. This sesquiterpene had significant repellent action against *S. zeamais* and persisted in the environment for 45 days at a dose of 10 L per 20 g corn grain.

Lawal *et al.* (2013) conducted chemical evaluation of mosquito repellent formulation prepared from the essential oil of leaves and peels of *Ocimum gratissimum*, *Cymbopogon citratus*, *Hyptis suaveolens*, *Eucalyptus globulus*, *Azadirachta indica* and *Citrus sinensis* using by Gas Chromatography (GC). A total of seven compounds were detected representing 99.92% of the essential oil components of the repellent formulation. The major component present was limonene (34.91%) and the other main components were salanin (4.34%), citral (22.64%), 1,8 - cineole (7.00%), citronella (17.49%), caryophyllene (11.25%) and azadirachtin (2.29%). The study showed the potential of volatile compounds of plants as possible constituents of repellent products and may therefore serve as an alternative to commercially available synthetic repellents.

Sinthusiri and Soonwera (2013) evaluated 20 herbal essential oils as insecticides against the housefly. Ten percent concentrations of *Cymbopogon citratus* (lemongrass), *Mentha piperita* (peppermint) and *Lavandula angustifolia* (lavender) oils were the most effective, showing 100% knockdown at 30 and 60 minutes. The KT50 values for *C. citratus*, *M. piperita* and *L. angustifolia* were 5.14, 5.36 and 8.23 minutes,

respectively. This study revealed that lemongrass, peppermint and lavender essential oils have the potential to control housefly populations.

Hanan (2013) evaluated the insecticidal activities of peppermint, *M. piperita* and lavender, *Lavandula angustifolia* essential oils against housefly for their larvicidal and pupicidal activities. *M. piperita* showed higher toxicity against *M. domestica* larvae than *L. angustifolia*. The LC₅₀ and LC₇₅ values were 2.5% (225 ppm) and 3% (270 ppm), respectively, for *M. piperita* and 3% (264 ppm) and 4% (352 ppm) respectively, for *L. angustifolia*. Moreover, a significant prolongation in both larval and pupal duration, reduction in pupation and adult emergency percent in addition to various morphological abnormalities of larvae and pupae were detected post treatment of the third larval instar with LC₅₀ and LC₇₅ of *M. piperita* and *L. angustifolia*. The results revealed that the essential oils of peppermint and lavender have a control potential against *M. domestica*.

Mansour and Mohamed (2013) evaluated the insecticidal property of herb *Conyza aegyptiaca* against mosquito, *Anopheles pharoensis* and *M. domestica*. LC₅₀ of the oil accounted to 37.8 ppm and 0.087 mg/cm² against larvae and adults of *An. pharoensis*, respectively. These toxicity parameters were found to be 71.8 ppm as LC₅₀ and 0.125 µg/insect as LD₅₀ against larvae and adults of *M. domestica*, respectively. It was concluded that the potency of *C. aegyptiaca* oil refers mainly to the presence of limonene.

Rossi and Palacios (2013) evaluated the fumigant toxicity of *Citrus sinensis* essential oil on *Musca domestica* adults in the absence and presence of a P450 inhibitor. In a fumigant assay, *M. domestica* adults treated with *Citrus sinensis* EO (LC₅₀ = 3.9

mg/dm³), with (4R)(+)-limonene (95.1%) being its main component, died within 15 min or less. They reported that *C. sinensis* EO, (4R)(+)-limonene, α -pinene and β -pinene are absorbed by flies exposed to it; consequently, this oil acts as a potent fumigant against *M. domestica*. Flies metabolize (4R)(+)-limonene into carveol and carvone; these metabolites showed less toxicity against *M. domestica* and this fact suggested that the fly uses the oxidation reaction for the detoxification of (4R)(+)-limonene. The toxicity of the EO and of (4R) (+)-limonene increased when a P450 inhibitor is used in combination with either of them, suggesting that P450 monooxygenase mediated this detoxification.

Manzoor *et al.* (2013) evaluated the acaricidal activity of essential oils from Tulsi (*O. basilicum*), Bach (*Acorus calamus*) and Mint (*Mentha arvensis*) against *R. sanguineus*. The highest acaricidal activity (100 % mortality) was observed with *O. basilicum* oil at 1:15 (66.6 μ L/mL) with LC₅₀ value as 32.31 μ L/mL. 100 % mortality was obtained for concentrations (66.6, 33.3, 22.2, 16.6 μ L/mL) at 60 min for *O. basilicum*.

Ahmed *et al.* (2013) examined the use of plant extracts viz., Niaz boo (*O. basilicum*) Gardenia (*Gardenia jasminoides*), Sanatha (*Dodonaea viscosa*) and Lantana (*Lantana camara*) as oviposition attractant and larval growth promoter/inhibitor on the house fly. *L. camara* was a strong repellent for oviposition and showed a significant larvicidal activity. *O. basilicum* was repellent for oviposition where least number of eggs were reared 92.56 eggs female⁻¹. They reported that decoction of leaves of *D. viscosa*, *L. camara* and *O. basilicum* proved effective for the management of flies, either as spray or in bait systems.

Karamaouna *et al.* (2013) evaluated the insecticidal activity of aromatic plants against vine mealybug, *Planococcus ficus* such as peppermint, *Mentha piperita*, thyme-leaved savory, *Satureja thymbra*, lavender, *Lavandula angustifolia*, and basil, *O.basilicum*, lemon, *Citrus limon*. Bioassays were conducted in the laboratory by using spray applications on grape leaves bearing clusters of *P. ficus* of one size class, which mainly represented either 3rd instar nymphs or pre-ovipositing adult females. The LC₅₀ values of basil oil were ranged from 44.1 to 46.8 mg/mL and basil essential oils were less toxic than the paraffin oil which was used as the reference product.

Zimmer *et al.* (2013) evaluated the larvicidal activity and sub lethal effects of entomopathogenic bacteria *Brevibacillus laterosporus*, *Bacillus thuringiensis var. israelensis*, *B. thuringiensis var. kurstaki*, and a commercial formulation of *B. sphaericus* on *Musca domestica*. Bacterial suspensions were prepared in different concentrations and added to the diet of newly-hatched larvae which were monitored until the adult stage. The larvae were susceptible to the *B. laterosporus*, *B. thuringiensis var. israelensis*, and *B. thuringiensis var. kurstaki* bacteria in varied concentration levels. These bacteria have larvicidal and sub lethal effects on the development of flies, reducing both adult size, and impairing the reproductive performance of the species.

Al-Olayan (2013) evaluated the pathogenicity of *Acremonium cephalosporium*, *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride*, and *Verticillium albo-atrum* mitosporic ascomycete fungi to the housefly, *M. domestica*. The results of the current study revealed that the fungi such as *A. niger*, *T. viride*, and *V. albo-atrum* were effective for the control of the house fly, *M. domestica* and could be used as biological control agents.

Machtinger and Geden (2013) evaluated the host location by *Spalangia cameroni* in equine associated substrates. Houseflies and stableflies were equally vulnerable to parasitism and none of the substrates tested fell outside the range of moisture and density properties. Innate preferences among these substrates were small and were overwhelmed when parasitoid abundance was high. It did not appear as though equine-generated substrates would negatively influence the success of filth fly parasitism by *S. cameroni*. Therefore, these results suggested that *S. cameroni* is a suitable candidate for augmentative releases for fly management programs on Florida horse farms.

Mwamburi *et al.* (2014) evaluated use of two formulations and two application techniques to deliver *B. thuringiensis var. israelensis* for the control of *M. domestica* larvae and adults in poultry houses. They reported that simultaneous use of Bti formulations (bran and WDG formulations) could increase the efficiency of the bacterial pathogenic agents and therefore allow a lower dose of their combination to be used for the control of housefly larvae.

Kiplangat and Mwangi (2014) reported that sweet basil oil served to paralyze mosquitoes and block them on the skin surface since it acts in a vapour-phase most likely due to camphor and limonene components of sweet basil which are known for repellent activity.

Ogbalu *et al.* (2014) evaluated the larvicidal effect of aqueous leaf extract of tobacco (*Nicotiana tabacum*) on the third instar larvae of *M. domestica* at the concentration of 1, 2, 3, 4, 5 milligram per litre where 1mg/l at 24hours showed [11.05%], using 2mg/l [72.6%], using 3mg/l [97.8%] mortality was observed. They

suggested that the aqueous leaf extracts of *N. tabacum* have the potential to be used as an eco-friendly approach for the control of maggots of *M. domestica*.

Bushra and Tariq (2014) reviewed several essential oils to control the mosquito species.

Mansour *et al.* (2014) evaluated the insecticidal activity of chicory (*Cichorium intybus*) extracts against two dipterous insect-disease vectors: Mosquito and housefly. Toxicity screening against larvae and adults of the mosquito (*Anopheles pharoensis*) and the housefly (*M. domestica*), revealed LC₅₀ of 15.3 mg kg⁻¹ and 0.023 mg/cm² against larvae and adults of mosquitoes, respectively. The LC₅₀ for housefly larvae equaled 65.8 mg kg⁻¹ and the LD₅₀ for adults equalled 0.112 g/insect. Isolation and identification of two biologically active compounds, e.g., lactucopicrin-15-oxalate (compound I) and chicoralexin (compound II); both showed high toxicity towards the two tested insects. Biologically active compounds, e.g., lactucopicrin-15-oxalate and chicoralexin identified; both showed high toxicity towards the two tested insects. The obtained results provide novel data focusing on the insecticidal efficacy of this native plant against pests of medical importance such as mosquitoes and housefly.

Rossi and Palacios (2014) evaluated the insecticidal toxicity of *Eucalyptus cinerea* essential oil and 1,8-cineole against *M. domestica*. In a fumigant assay, *M. domestica* adults were treated with *E. cinerea* EO [LC₅₀ = 5.5 mg/dm³). One of the majority component is 1,8-cineole α -pinene and α -terpineol were absorbed by flies exposed to it and consequently this oil acted as a potent fumigant against *M. domestica*. They reported that 1,8-cineole acts as a P450 inhibitor-like compound, improving the

toxicity of other xenobiotics such as deltamethrin. The use of the *E. cinerea* EO and or 1,8-cineole as fumigants or as synergistic of pyrethroid insecticides is an interesting alternative to control flies in human habitats, especially against resistant flies, being able to decrease the doses of synthetic insecticides.

El-Sherbini and Hanykamel (2014) tested the efficacy of *Fortunella crassifolia* swingle peel with insecticidal activity against housefly. The essential oil showed potent insecticidal activity.

Soonwera and Sinthusiri (2014) evaluated the Thai essential oils of *Syzygium aromaticum* (Clove) and *Cymbopogon citratus* (lemongrass) for their larvicidal, pupicidal, adulticidal, oviposition deterrent and ovicidal activities against house fly. The highest insecticidal activity was shown by clove oil with LC₅₀ values of 1.20-10.55% and exhibited highest oviposition deterrent and ovicidal activities with 100% effective repellency and 0% of hatching rate. These results exhibited higher toxicity than cypermethrin and lemongrass oil.

Soonwera (2015) evaluated the larvicidal and oviposition deterrent activities of Ylang ylang (*Cananga odorata*), citronella grass (*Cymbopogon nardus*) and clove (*Syzygium aromaticum*). The highest larvicidal activity and oviposition deterrent activity was shown by *S. aromaticum* oil with LC₅₀ values of 9.83% and 100% effective repellency and -1.0 OAI at 1.65µl/cm². *S. aromaticum* oil also exhibited the higher larvicidal and oviposition deterrent activity than cypermethrin. Toxicity assay indicated the order of LC₅₀ values and percentage of effective repellency against housefly in three essential oils as *S. aromaticum oil* > *C. odorata oil* > *C. nardus oil*. These results clearly

revealed that *S. aromaticum* oil served as green pesticide to control house fly population and was safe for humans and the environment.

Bobi *et al.* (2015) evaluated insecticidal efficacy of ethanol extract of leaf of *Azadiracta indica*, *Calotropis procera*, *Ocimum basilicum* and *Argemone mexicana* against various developmental stages of *Musca domestica* in the laboratory. Dipping and feeding methods were employed to evaluate the insecticidal potentials of the plant extracts at five different concentrations (100 ppm, 200 ppm, 300 and 400 ppm) of the extracts. All the selected plant extracts were found to affect the pupation and emergence of adults ($p < 0.001$).

Zibae and Khorram (2015) investigated the synergistic effect of *Eucalyptus globulus*, *Rosmarinus officinalis* essential oils and their mixed formulation on cockroaches, mosquitos and housefly where all essential oils were toxic and showed adulticidal activity.

Eliopoulos *et al.* (2015) studied the fumigant activity of essential oil vapors distilled from sweet basil *O. basilicum* against two major stored product pests viz. *Ephestia kuehniella* (Zeller) and *Plodia interpunctella* Hübner, 1813. They reported that the oil was highly effective against adults but less effective against immature stages (larva and pupa).

Mohamed (2015) reported the toxicity of Basil (*Ocimum basilicum* L.) extracts on *Tribolium confusum* (Coleoptera: Teneberionidae) and found that the last larval instar was more highly susceptible to both plant extracts than the adult stages. The LC_{50} of

O. basilicum extract were 148.38 and 218.78 ppm for the last larval and adult stages after 24 hours exposure, respectively. They reported that the application of these botanical extracts might be promising in protecting the stored grains against coleopteran pests.

Abiy *et al.* (2015) evaluated the repellent efficacy of DEET, MyggA, neem (*A. indica*) oil and chinaberry (*Melia azedarach*) oil against *Anopheles arabiensi* in Ethiopia. DEET and MyggA provided more than 96% protection. The mean protection time for DEET was 8 hrs while the time for MyggA was 6 hrs. Protection obtained from neem oil and chinaberry oil was almost similar (more than 70%), however, the complete protection time for neem was 3 hrs, while that of chinaberry oil was one hour.

Pinto *et al.* (2015b) investigated the chemical composition and insecticidal activity of *Cymbopogon citratus* essential oil from Cuba and Brazil against housefly. The essential oils were applied topically to newly-hatched larvae (1 μ L/larva). The results showed a lethal concentration (LC₅₀) of 4.25 and 3.24% for the Brazilian and Cuban essential oils, respectively demonstrating its potential insecticidal activity.

Buentello-Wong *et al.* (2016) tested the toxicity of *Eugenia caryophyllus*, *Ocimum basilicum* and *Thymus vulgaris* against adults of the fruit pest the Mexican fruitfly, *Anastrepha ludens* (Diptera: Tephritidae). In ingestion toxicity assays, the EO of *E. caryophyllus* was the most toxic, with an LC₅₀ of 3529 ppm, followed by the EOs of *T. vulgaris* and *O. basilicum* with LC₅₀s of 5347 and 8050 ppm, respectively. At the highest concentration (1.5, 2.0 or 3.5% w/v), 100% mortality was observed with the three EOs in the ingestion toxicity assays. In general, the three EOs were significantly toxic to

adult *A. ludens*. The development of a technology to incorporate the EOs into food bait could provide an alternative method to attract and kill *A. ludens* in field applications.

Nurulain *et al.* (1994) studied the toxic effect of crude neem seed kernel extract (RB-a) against *M. domestica* L. (PCSIR Strain) and found it to be less effective than synergistic formulation, prepared with PBO, and Tx-100 was found to be 144 times more effective than RB-a alone. LD₅₀ of RB-a formulation was determined as 5.5 ug/fly while LD₅₀ of synergistic formulation of RB-a was about 800 µg/fly.

2.6 Evaluation of biocontrol agents such as entomopathogenic nematodes for fly control

Chemical control of house fly has its own limitations. Therefore, identification of alternative strategies to manage this important pest by using more eco-friendly methods such as use of biological agents (bacteria, virus, fungi, entomopathogenic nematodes) are of paramount importance for the development of successful control against the housefly. Therefore the following literature has been reviewed with respect to biological control.

2.6.1 In India

Malik *et al.* (2007) reviewed control strategies for housefly mainly biological control methods that comprise botanical, fungal, bacterial, entomopathogenic nematodes and parasitoid agents.

Yadav and Lalramliana (2012) evaluated the efficacy of three indigenous strains of entomopathogenic nematodes *H. indica*, *S. thermophilum* and *S. glaseri* against mustard sawfly, *Athalia lugens proxima* Klug and reported that last instar larva of

mustard sawfly were found to be susceptible to all the three EPNs tested, but the degree of susceptibility to infection varied among nematode species. Based on LC_{50} value, *H. indica* was the most pathogenic species. Their study revealed that all the three test nematodes were also able to propagate in the host cadaver and produce first generation infective juveniles. However, *H. indica* produced significantly more number of IJs per insect larva than the other two nematode species. The progeny production was recorded to be the least in case of *S. glaseri*.

Yadav and Lalramliana (2012) studied about the reproduction of entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyx chalybaeus*. They found that *H. indica* reproduced more compared to *S. thermophilum* and *S. glaseri*.

Patil *et al.* (2015) did a study on synergism of entomopathogenic nematode and imidacloprid on early and late 3rd instars of coconut white grub, *Leucopholis conioophora* Burmeister, 1855 and reported that different imidacloprid concentrations, exposure time and interaction between imidacloprid and exposure time did not affect nematode mortality in bioassay.

2.6.2 In different parts of world

Geden *et al.* (1986) studied the infectivity of the *S. feltiae*, *S. glaseri* and *H. heliothidis* for the larval, pupal and adult stages of the house fly under different habitat conditions. Second and 3rd-instar larvae and adult flies were highly susceptible to *S. feltiae* and *H. heliothidis* when hosts were confined in petri dishes containing nematode-treated filter paper. Larvae were not susceptible to *S. glaseri* and pupae within

puparia were refractory to infection by all 3 species. When 2nd and 3rd-instar larvae were exposed to nematodes in rearing medium, *S. feltiae* caused higher mortality (55-61 %) than *H. heliothidis* (11-26%) at the highest dosage of 5,000 nematodes per host. Both *S. feltiae* and *H. heliothidis* were more infective for 3rd-instar larvae (21-29%) than for 2nd-instar larvae (2-6%) at this dosage in poultry manure.

Belton, *et al.* (1987) reported *H. heliothidis* as a potential biological control agent of house flies in caged-layer poultry barns as application of *H. heliothidis* to manure can significantly decrease the number of adult flies.

Georgis *et al.* (1987) studied the survival, infectivity and movement of three insect parasitic nematodes (*S. feltiae*, *S. bibionis*, and *H. heliothidis*) in poultry manure under laboratory conditions. They found that the majority (70-100%) of the nematodes died within 18 hours after exposure to the manure. Nematodes exposed to manure slurry for 6 hours killed at least 95% of the house fly larvae, *M. domestica*, but nematodes exposed for 12 hours achieved less than 40% larval mortality. The majority (90-97%) of the three nematode species applied to the manure remained on the surface. Poor survival and limited movement of nematodes in poultry manure appear to make them unlikely candidates for biocontrol of filth flies in this habitat.

Mullens *et al.* (1987) evaluated the infectivity of insect-parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against third-instar larvae of *M. domestica*, *O. aenescens*, *F. canicularis*, and *F. femoralis* in the laboratory. Four strains of *S. feltiae*, two of *S. bibionis*, and one of *H. heliothidis* were tested on moist filter paper in petri dishes. Considerable variability in infectivity within and among hosts

was noted for the different nematode strains. Strains of *S. feltiae* were more infective than *S. bibionis* or *H. heliothidis*, but all could infect the four hosts. *F. femoralis* was highly resistant to infection by *S. bibionis* and *H. heliothidis*. Further tests on chicken manure and fly rearing medium showed that infectivity of several nematodes for *M. domestica* and *F. canicularis* larvae was markedly less than on filter paper.

Renn (1995) encapsulated entomopathogenic nematodes in calcium alginate to test mortality against housefly in artificial diet and chicken manure and demonstrated that it was possible to control animal house infestations of *M. domestica* by applying either *S. feltiae* or *H. megidis* to the manure in a formulation which allows their slow release and which offers some protection for the nematodes.

Hsiao *et al.* (1997) reported the impact of animal manure on survival and pathogenicity of the *S. carpocapsae* where they reported that poultry, swine, beef and cattle manure were inhibitory to the *S. carpocapsae* as they significantly lowered the number of nematodes extracted from manure-amended soils.

Renn (1998) reported various routes of penetration of the entomopathogenic nematode *S. feltiae* attacking larval and adult houseflies where it was found that *S. feltiae* preferentially penetrated housefly larvae via the anus and the cloaca in adults.

Taylor *et al.* (1998) studied the susceptibility of housefly larvae to entomopathogenic nematodes in cattle feedlots. They screened 40 strains representing 8 species of *Heterorhabditis* and 5 species of *Steinernema* for virulence toward 3rd-instar maggots. None of the 22 strains of *Heterorhabditis* infecting maggots caused significant

levels of mortality in a filter paper assay. Ten strains of *Steinernema* infected maggots, of which 7 strains (4 *S. carpocapsae*, 2 *S. feltiae*, and 1 *S. scapterisci*) caused significant mortality. Ten *Heterorhabditis* strains and 10 *Steinernema* strains successfully reproduced for ≥ 2 generations in maggots. Two strains of *S. feltiae*, SN and UNK-36, and 2 of the best *Heterorhabditis* strains, *H. bacteriophora* OSWEGO and *H. megidis*, HF-85 were tested in a fresh bovine manure substrate. All 4 strains produced significant fly mortality in the manure substrate, although the *S. feltiae* strains had significantly lower LC₅₀ values than did the *Heterorhabditis* spp. The most promising strain, *S. feltiae* SN gave LC₅₀ and LC₉₉ values of 4 and 82 infective juveniles per maggot, respectively. These doses were equivalent to 2.7 and 55 infective juveniles per gram of manure and 5.1 and 104 infective juveniles per square centimeter of surface area.

Mahmoud *et al.* (2007) evaluated the efficacy of the entomopathogenic nematode *S. feltiae* cross n 33 against larvae and pupae of four fly species in the laboratory. Mortality of second and third instar maggots in all fly species increased significantly with the increase of *S. feltiae* concentration and time post-application. *L. sericata* was the most susceptible second instar to *S. feltiae* with LC₅₀ and LC₉₀ of 47.04 and 193.8 IJs, respectively. *M. domestica* had the most susceptible third instar maggots with LC₅₀ and LC₉₀ of 75.1 and 292 IJs, respectively. In soil and manure, the rates of infected pupae and fly emergence differed significantly among the tested concentrations within each fly species. Rates of infected pupae and LC₅₀ and LC₉₀ were consistently higher in soil than in manure.

Geden (2012) reviewed the biopesticides such as entomopathogenic nematodes, fungi, bacteria, virus, and botanicals for control of house flies.

Iqbal *et al.* (2014) reviewed the disease vector potential of housefly and integrated control of housefly by different methods including various biological agents. They stated that entomopathogenic nematodes did not show better results in pig and poultry manure.

Sharifi *et al.* (2014) tested the efficacy and reproduction of two entomopathogenic nematode species as potential biocontrol agents against the rosaceae longhorned beetle, *Osphranteria coerulescens* Redtenbacher, 1850. Both nematode species were able to penetrate and reproduce within *O. coerulescens* larvae and reported that the reproduction rate for *H. bacteriophora* was higher than *S. carpocapsae* suggesting reproduction of Heterorhabditids was more compared to Steinernematids.

2.7 Evaluation of agripower diatomite (diatomaceous earth) for control of flies

Diatomaceous earth (DE) is pure amorphous silicon dioxide made up of fossilised diatoms; it acts as an insecticide by absorption of epicuticular lipids and fatty acids, leading to desiccation in arthropods. Therefore the following literature has been reviewed with respect to DE.

2.7.1 In India

Rajasri *et al.* (2014) tested inert dusts for the management of angoumois grain moth, *Sitotroga cerealella* Olivier, 1789 in stored rice. Diatomaceous earth @ 2.5g and 5 g /kg seed were evaluated. DE @ 5g/kg were found to be effective against *S. cerealella* upto twelve months of storage of rice with less insect damage (<0.5%) compared to deltamethrin (6.38%) and untreated control (8.73%). These eco-friendly inert dusts were

recommended as seed protectants to save the rice seed against stored grain pests for longer periods.

2.7.2 In different parts of world

Arthur (2000) evaluated the toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae). Insects were exposed to the diatomaceous earth at 0.5 mg/cm² on filter paper inside plastic petri dishes. Mortality of both species after they were held for 1 wk was greater than initial mortality for nearly all exposure intervals at each temperature-humidity combination, indicating delayed toxic effects from exposure to diatomaceous earth.

Arnaud *et al.* (2005) evaluated the efficacy of diatomaceous earth formulations admixed with grain against populations of *Tribolium castaneum*. Four commercially available DE formulations were tested: INSECTOs, Perma-Guardt, Protect-Its and the diatomite used for the production of Dry acides, each at six concentrations (100–1000 ppm). A great variation of efficacy was observed among the DE formulations tested. It was found that some populations can be satisfactorily controlled with some DE formulations but not with others.

Ziaee *et al.* (2007) evaluated the efficacy of Silicosec® a diatomaceous earth formulation against *T. castaneum* (Coleoptera:Tenebrionidae) against 7-14day old adult, old and young larvae. Results indicated that mortality of *T. castaneum* young larvae of red floor beetle were more sensitive to Silicosec than old larvae and adults. Reproductive potential of adults in treated wheat was suppressed when compared to untreated wheat.

Athanassiou *et al.* (2007) investigated the insecticidal effect of three diatomaceous earth formulations, applied alone or in combination, against three stored-product beetle species on wheat and maize. For all species tested, all DEs were more effective on wheat than on maize. Generally, the mix of two or three DEs was more effective than the application of one DE, for all species and commodities. The results of the work clearly indicated that a blending of several DEs together may produce a new DE formulation that is highly effective at low dose rates.

Kabir *et al.* (2011) evaluated the efficacy of raw diatomaceous earth against *T. castaneum* (Coleoptera: Tenebrionidae) on stored maize, sorghum and wheat. 30 adults were exposed to 50g grain sample with diatomaceous earth at 125, 250, 500, 1000 and 1500 ppm. At 14 days of exposure 100% mortality was observed in wheat and maize at 500 and 1000ppm respectively, where as only 72% in sorghum at 1500ppm. Progeny production was suppressed in all DE treated compared to control. The results indicated that it was possible to control *T. castaneum* using raw DE.

Jean *et al.* (2015) evaluated the efficacy of diatomaceous earth for the control of *Sitophilus zeamais* in stored maize. Maize grains were admixed with the dusts at the rates 0, 0.5, 1, 1.5 and 2 g/kg for FossilShield. At 0.5 g/kg achieved 100% mortality of *S. zeamais* within 7 days of exposure. The dust caused significant reduction of progeny emergence, damaged grains and weight loss, but showed no adverse effect on the viability of the protected seeds. Fossil Shield could be of value as green stored grain protectant against *S. zeamais*.

Ofuya *et al.* (2015) evaluated diatomaceous earth for the management of *Callosobruchus maculatus* Fabricius, 1775 in stored cowpea. Treated at 0.02, 0.04, 0.06, 0.08 g/20g of seed. All the application rates of the diatomaceous earth produced significant mortality of *C. maculatus* in 24 hours (85.6-90.0%); and in 48 hours (100.0%) post-treatment compared with the control. Therefore it had a potential of controlling *C. maculatus* in stored cowpea.

Shiell (2015) evaluated the effectiveness of naturally occurring substances in diatomaceous earth at reducing house fly reproduction in duck manure. It was added at 1.9 and 4.7% to 200g duck manure. Diatomaceous earth was added to duck manure at the concentrations tested (1.9 or 4.7%) and did not significantly reduce adult house fly emergence or repel flies from landing compared to untreated manure.

Materials and Methods



III. MATERIALS AND METHODS

3.1 Prevalence and diversity of fly infestation in poultry farms

3.1.1 Study area

Ten different poultry farms in Bangalore urban and rural districts and one farm in Davangere district were selected and flies were collected by using sweep net (Fig.1) and larvae were collected by hand picking from poultry manure (Fig. 2). Collections were made during three different seasons, *viz.*, rainy season (south west monsoon: June, July, August and September: north west monsoon: October, November and December), winter season (January, February) and summer season (March, April and May) during 2013 and 2014 from different locations and rearing conditions as detailed below in (Table 1).

Table 1: Particulars of collection of flies

Sl. No	Places of collection	Type of rearing
1.	Pragathi Poultry Farm, Nelmangala, Bangalore rural	Cage rearing
2.	Venkateshwara Breeding Farm, Kannur, Bangalore East	Cage rearing
3.	Monarca Hatcheries, Honnenahalli, Bangalore North	Cage rearing
4.	Rajeshwari Poultry farm, Hoskote, Bangalore rural	Cage rearing
5.	Thangaraju Poultry Farm, Hancharahalli, Bangalore East	Deep litter
6.	CPF Poutry Farm, Mandur, Bangalore East	Cage rearing
7.	State Poultry Farm, Hessarghatta, Bangalore rural	Deep litter
8.	Jagadeesh Poultry Farm, Hessarghatta, Bangalore rural	Cage rearing
9.	KVAFSU Poultry Farm, Hebbal, Bangalore	Cage rearing, deep litter
10.	Bannerghatta Poultry Farm, Bangalore rural	Cage rearing
11.	Davangere Poultry Farm, Dhavangere	Cage rearing



Fig. 1: Sweep net for collection of flies



Fig. 2: House fly larvae in poultry manure

3.1.2 Collection, separation and storage

3.1.2.1 Adult flies

- Adult flies were collected by using sweep net
- Different flies were preliminarily identified based on wing pattern, color of flies, head and thorax pattern and separated.
- Some adult flies were dry preserved to preserve their natural colour and bristles, and the rest were preserved in 70% ethanol. The date of collection, season, species, region of collection was recorded.
- Of the different flies some were stored in 95% ethanol at -20°C in the deep freezer for molecular studies.

3.1.2.2 Larvae

- Larvae were collected by handpicking from poultry manure.
- Larvae of different flies were separated and stored in 70% ethanol. Date of collection, season, species and region of collection was recorded.
- Of the different larvae separated some were taken and stored in 95% ethanol at -20°C in the deep freezer for molecular studies.

3.2 Clearing of adult flies and larvae by carbolic acid (liquified phenol) was done as per the method of Wirth and Martson (1967) as detailed below

1. Liquified phenol was prepared by diluting pure phenol (carbolic acid) crystals with enough absolute (100%) ethyl alcohol to form a saturated solution with a layer of

about ½ by volume of phenol crystals in the bottom of the container. This solution was prepared when required and used freshly.

2. A bottle of phenol-balsam mixture was prepared by mixing equal parts (v/v) of liquified phenol and Canada balsam of the consistency normally used for making slides or a little thicker. This mixture was prepared one day in advance of use and not more than 3 weeks ahead, since it tended to become dark.

3.2.1 Procedure for clearing

Liquified phenol was placed in a cavity slide and the specimens were transferred to it and was left in the solution until they were cleared.

3.2.2 Slide preparation

A drop of phenol-balsam mixture was placed on a microscope slide. Then the specimen from the phenol solution was transferred to the slide. The specimen was dissected and separated part by part, such as head, wing, thorax, abdomen and legs where as in case of larvae, anterior and posterior parts were cut for mounting. Then a cover slip was placed on it. It was then labelled with details such as species, date and place of collection and kept for drying. Later the specimens were morphologically identified.

3.3 Morphological identification

Morphological identification of adult flies was done by using keys of White *et al.*, 1940; Sabrosky, 1949; James, 1960; Van Emden, 1965; Zumpt, 1965; Fonsseca 1968; Walker, 1994; Triplehorn and Johnson, 2005; Carvalho and Patiu, 2008; Whitworth 2010a and b; Lin *et al.*, 2010; Szpila, 2010; Meiklejohn, 2012; Ramaraj, *et al.* 2014; Irish

et al. 2014; Akbarzadeh *et al.*, 2015; Oliveira *et al.* 2015. The characters for morphological identification of fly included length of fly, eyes, antennae, wing pattern, and colour of flies, thorax and abdominal pattern.

Morphological identification of larvae were done by using keys of Zumpt, 1965; Holloway 1991; Wells *et al.*, 1999; Sukontason *et al.* 2003a and b; Sukontason *et al.*, 2004a; Brink 2009; Thyssen, 2010; Szpila 2010, Velasquez *et al.* 2010. Basic morphological features of larva used for identification purpose included length of larvae, integument, spines, cephalopharyngeal skeleton, respiratory structures such as anterior and posterior spiracles.

3.3.1 Micrometry

The micrometer was calibrated for different magnifications such as 4X and length of adult flies, egg and larvae were taken.

3.4 Genomic studies

3.4.1 Extraction of Genomic DNA

3.4.1.1 Materials

- a. Microcentrifuge tubes (Tarsons)
- b. Micropipettes (Tarsons)
- c. Microcentrifuge (Tarsons)

The genomic DNA of adult flies and larvae were extracted as per the standard protocol using the “DNeasy Blood and Tissue kit” procured from J J BIOTECH, Bangalore. Quiagen, Germany (manufacturers).

List of materials provided in the kit

DNeasy Mini Spin Columns	50 Nos.
Collection tubes (2ml)	150 Nos.
Buffer ATL (Tissue lysis buffer)	10ml
Buffer AL (Lysis buffer)	12ml
Buffer AW1 (Concentrate)	19ml
Buffer AW2 (Concentrate)	13ml
Buffer AE	22ml
Proteinase K	1.25ml

3.4.1.2 Procedure for extraction of DNA

- Individual fly specimen was taken, from whole fly head, wings, legs and thorax were removed and only abdominal part was used for DNA extraction. Later 180µl of buffer ATL was added and the pestle was fixed to the motor and homogenised gently for 2min.
- 20µl of Proteinase K was added to the above and mixed thoroughly by vortexing for 15sec and incubated at 56°C for overnight.
- 200µl buffer AL was added to the above and mixed thoroughly by vortexing for 15 sec. and incubated at 56°C for 10 min.
- 200µl of ethanol (100%) was added to the above and mixed thoroughly by vortexing for 15 secs.

- The whole mixture was pipetted into a DNeasy Mini Spin column placed in a 2ml collection tube. It was centrifuged at 6000xg (8000rpm) for 1min. The flow-through and collection tubes were discarded.
- The Spin column was placed in a new 2ml collection tube and 500µl of buffer AW1 was added. It was centrifuged for 1min at 6000xg (8000rpm). The flow-through and collection tube were discarded.
- The spin column was placed in a new 2ml collection tube and 500µl buffer AW2 was added. It was centrifuged for 3min at 20,000xg (14,000rpm). The flow-through and collection tube were discarded.
- The spin column was transferred to a new 2ml microcentrifuge tube.
- The DNA was eluted by adding 200µl buffer AE to the centre of the spin column membrane. It was incubated for 1min at room temperature (15-25°C) and centrifuged for 1min at 6000xg (8000rpm).
- Later DNA was stored at -20⁰C

3.4.2 DNA confirmation by agarose gel electrophoresis

Equipments

- a. Weighing balance (KERN)
- b. Horizontal electrophoresis apparatus with power pack (GENETIX)
- c. Microwave oven (LG)
- d. Gel documentation unit (DNR MiniLumi)
- e. Agarose (HIMEDIA)

Tris-borate EDTA buffer (TBE buffer) (10X, Ph8)

Tris base 10.8 g

Boric acid 5.5g

EDTA (0.5 M Ph8) 4ml

Distilled water up to 100ml

The stock solution was sterilized by autoclaving before use.

To make 1X TBE buffer, 10X buffer and distilled water added in the ratio of 1:20

To make 0.5X TBE take 25ml of 10X TBE and make up the volume to 500ml.

Gel loading dye (6X)

Bromophenol blue 0.25% (w/v)

Xylene cyanol 0.25% (w/v)

Sucrose 40% (w/v) in distilled water

Ethidium bromide (10mg/ml)

Ethidium bromide 100mg

Double distilled water 10ml

The suspension was stirred to ensure that the dye had dissolved. The container was then wrapped in aluminium foil.

3.4.2.1 Gel preparation for DNA check (0.8%)

About 0.8g of agarose (analytical grade) was dissolved in 95ml of distilled water and 5ml of 10X TBE buffer and melted in micro-oven for 2min until a clear uniform

suspension was obtained. Prior to casting the gel, the molten agarose was allowed to cool to about 50⁰C, after which 2ul of Ethidium bromide was added and mixed thoroughly. Gel was cast on the gel casting tray fitted with acrylic comb and left for setting. The acrylic comb was carefully removed after the gel was set. The tray with gel was submerged in an electrophoresis tank containing 0.5X TBE buffer.

5μl of DNA to be analyzed was mixed with 2μl of 6X DNA loading dye and charged into wells. Electrophoresis was carried until the tracking dye just reached the end of gel. Following the electrophoresis, DNA bands were visualized and the images were captured by using Gel Doc unit (DNR MiniLumi).

3.5 Molecular identification of flies-Barcoding

The morphologically identified flies were confirmed by molecular identification.

3.5.1 Polymerase Chain Reaction (PCR)

Morphologically six different flies were identified viz *Musca domestica*, *Chrysomya megacephala*, *Hydrotaea aenescens*, *Hydrotaea capensis*, *Hermetia illucens* and *Sarcophaga ruficornis*. Different flies from all the farms were subjected for barcoding. For barcoding, cytochrome oxidase subunit I mitochondrial gene was targeted and amplified by using universal primers.

Reagents

- a. 10X Taq buffer (Genei)
- b. dNTP (deoxy nucleoside triphosphate mix) (Genei)
- c. Taq DNA polymerase 1unit/ul (Genei)

- d. Sterile nuclease free water (NFW) (HIMEDIA)
- e. Primers (BIOSERVE)
- f. Template DNA extracted from fly
- g. 100 bp DNA ladder (HIMEDIA)

3.5.2 PCR amplification of COI (mitochondrial gene) for barcoding of different flies

COI gene of flies and larvae were amplified by universal primers LCO1490/HCO2198. The published universal primers were synthesized by Bioserve Biotechnologies (India) Pvt. Ltd, Hyderabad. The details of the primers and their base sequences are detailed below (Table 2). The primers obtained were reconstituted in nuclease free water (NFW) as per the requirements and stored at -20°C.

Table 2. Nucleotide sequence of LCO1490/HCO2198 primers

Primer code	Nucleotide sequence	Product size (bp)	Reference
LCO1490 HCO2198	5'-GGTCAACAAATCATAAAGATATTGG-3' 5'-TAAACTTCAGGGTGACCAAAAATCA-3'	658	Hebert <i>et al.</i> (2003), Rivera and Currie (2009), Hamada <i>et al.</i> (2010), Pramual <i>et al.</i> (2011) and Puente. <i>et al.</i> (2012), Gutierrez <i>et al.</i> (2014), Bhaskaran and Sebastian (2015) etc

Table 3: Composition of PCR mix for species specific amplification of COI of flies

Contents	Quantity
10X Taq buffer	5 μ l (Mgcl ₂ -15mM)
dNTP's mix	1 μ l (2.5mM each)
Taq DNA polymerase (1unit/ul)	1 μ l (1unit)
LCO1490	2 μ l (20pm)
HCO2198	2 μ l (20pm)
Template	5 μ l
NFW to make final volume	50 μ l

The amplification reactions were carried out in 0.2ml PCR tubes using a programmable thermal cycler using the following cycling conditions. (With slight modification of Hebert *et al.*, 2003), (Table 4).

Table 4: PCR conditions for the amplification of COI of fly spp.

Initial denaturation	Denaturation	Annealing	Extension	Final extension	Stop reaction
95°C 5min	95°C 1min	45°C 1min	72°C 1min	72°C 10min	4°C 5min
Repeated for 30 cycles					

After completion of PCR reaction, 5 μ l of amplified products along with 6X gel loading dye were subjected to electrophoresis in 1.5% agarose gel and 100 bp DNA ladder was used as marker. The images were captured using gel documentation system.

3.5.3 Sequencing of PCR products

The PCR products were sent to Euro Fins Ltd. Bangalore, India, for sequencing, where PCR products were sequenced in both forward as well as reverse directions. Sequences were then checked for homology by using bioinformatics tool BLAST (Basic local alignment search tool) from NCBI (National centre for Biotechnology information) server.

3.6 Evaluation of bio control agents such as entomopathogenic nematodes for fly control

3.6.1 Insect culture

Houseflies were initially collected from poultry house located in Veterinary College, Bengaluru, Karnataka and were identified based on the morphological keys developed by Zumpt, 1965; Van Emden, 1965 and Fonsseca, 1968. Adult flies were provided with milk and granulated sucrose. Then the flies were transferred to artificial diet to lay eggs. Eggs were collected and then transferred to fresh artificial diet. Larvae were reared on artificial diet. Fresh eggs were collected to test their susceptibility to EPNs while 2nd, 3rd instar and pupal stages were collected after 2, 3, and 5 days after introduction of eggs to the artificial diet, respectively (Geden *et al.*, 1986).

3.6.2 Nematode culture

The entomopathogenic nematodes *Heterorhabditis indica*, *Steinernema carpocapsae*, *S. glasseri*, *S. abbasi* and *S. feltiae* used in this study were collected from the live nematode culture of the Nematology laboratory, ICAR-National Bureau of Agricultural Insect Resources (NBAIR) Bengaluru, India and were maintained on late

instar larvae of greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) at 25 ± 2°C, using the method described by (Woodring and Kaya, 1988). Nematode infective juveniles (IJ) emerging from the *G. mellonella* larvae within 3 days from the first day of emergence were collected using White traps. Nematode viability was 100%, unless otherwise stated. In all the experiments the fresh infective juveniles (IJs) were used.

3.7 Nematode survival

Approximately 50,000 IJs of each *H. indica*, *S. carpocapsae*, *S. glasseri*, *S. abbasi* and *S. feltiae* nematode species in 1 ml water were applied to each of 16 petri dishes (5.0 cm), containing poultry manure (Fig. 3). Manure had been field-collected and heat killed for any existing arthropods. After introduction of IJs, dishes were arranged in a completely randomised design with four replicates. At 24 h intervals, four petri dishes were randomly selected to determine the survival of nematodes. Four manure samples were removed from each dish and placed in another dish containing 20 ml water (Georgis *et al.*, 1987). The IJs were separated from manure by pouring the suspension through a sieve followed by an additional 500 ml of tap water. The IJs were washed from the sieve into clean flask and re-suspended in 10 ml of sterilized water. To assess the nematode mortality after 24 h, 48 h, 72 h, 96 h, 200 µl samples were taken from each treatment and placed in a 100-mm petri dish lid to count the number of living and dead nematodes by using dissecting microscope. Immobile IJs were touched with a probe and considered dead if they did not react. The IJs were washed 4 times in sterilized, distilled water. The pathogenicity and reproducibility of nematodes was determined by adding 1 ml of suspension containing 400 IJs and two wax moth larvae to a 5cm petri dish lined with Whatman paper (Patil *et al.*, 2015). There were 4 petri dishes per treatment. Wax moth larval mortality was observed after 2 days. Dead larvae were removed and kept for

incubation for 10 and 7 days for *Heterorhabditis* and *Steinernema*, respectively, after incubation kept for emergence of IJs by keeping white traps for checking the reproduction.

3.8 Plate assay

3.8.1 Susceptibility of fly egg and pupa on filter paper

The efficacy of *H. indica*, *S. carpocapsae*, *S. glasseri*, *S. abbasi* and *S. feltiae* were tested against house fly eggs by placing 4 eggs in a petri dish (5.0cm) lined with a filter paper and adding 1 ml of a nematode suspension in distilled water at dosage levels of 0, 5,000 and 10,000 nematodes per egg (Fig. 4). Control treatments received only distilled water. Dishes were sealed with parafilm to avoid dehydration and kept at $25 \pm 2^\circ\text{C}$. There were 4 replicates for each nematode dose and petri dishes were placed in a completely random design. Egg mortality was checked based on larval emergence. The same protocol was followed for pupa except that mortality was based on adult fly emergence (Fig. 5).

3.8.2 Susceptibility of fly larvae on filter paper

To determine the pathogenicity of EPNs against second and third instars of housefly larvae, experiment was conducted in petri dishes (5.0 cm diam.) lined with a filter paper. Further, four second and third instar house fly larvae were placed in each petri dishes separately. Petri dishes were inoculated with the treatments at 7 levels: 50, 100, 200, 400, 800, 1600 and 3000 IJ larva⁻¹ and control received only distilled water. The containers were sealed with parafilm to avoid dehydration and kept at $25 \pm 2^\circ\text{C}$. There were 4 replicates per treatment in a completely random design (Fig. 6). Plastic

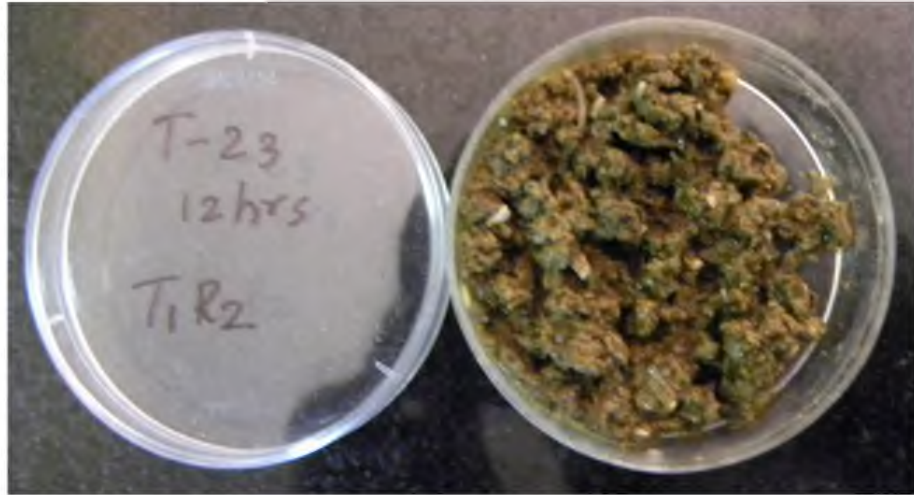


Fig. 3: EPN treated poultry manure for testing its survival

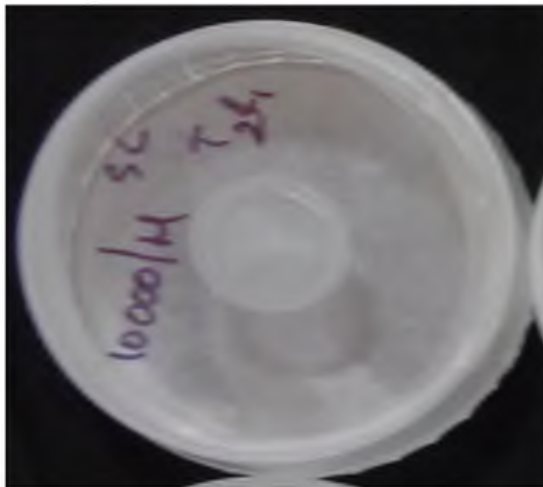


Fig. 4: Susceptibility of *M. domestica* eggs on filter paper



Fig. 5: Susceptibility of *M. domestica* pupae on filter paper

dishes were examined at 1, 2 and 3 days after treatment (DAT) and mortality was recorded. Cadavers were examined for signs of nematode infection (i.e., coloration) (Woodring & Kaya, 1988). Dead larvae were kept on white traps to observe nematode emergence from nematode killed insects. The same protocol was followed for 3rd-instar larvae except that mortality was based on adult fly emergence (observations were taken upto 5DAT).

3.8.3 Susceptibility of fly larvae in rearing medium

The same protocol as above was followed for second and third instar larvae except that the larvae were provided with artificial rearing medium (wheat bran and milk powder in the ratio of 8:2) (Fig. 7) and the mortality was based on adult fly emergence (observations were taken upto 5 DAT). In this experiment, artificial diet was provided to the larvae to note the differences in susceptibility, as the presence of food to larvae decreases the susceptibility of larvae to EPNs (Geden *et al.*, 1986).

3.8.4 Susceptibility of fly larvae in poultry manure

To determine the pathogenicity of EPNs against second and third larval stages of house fly larvae, experiment was conducted in plastic containers (5.8 cm diam., 200 cm³ soil capacity). Plastic containers were filled with 150 cm³ autoclaved manure (Fig. 8). Manure moisture was maintained up to the field capacity level. Further, four second and third stages of housefly larvae were placed in each plastic container separately. Plastic containers were inoculated with the treatments: 50, 100, 200, 400, 800, 1600, 3000, 6000, 7000, 8000, 9000, 10000, 12000, 14000, 16000, 18000, 20000, 32000, 64000, 128000 and 256000 IJs/larva and distilled water for control. There were 4 replicates per treatment

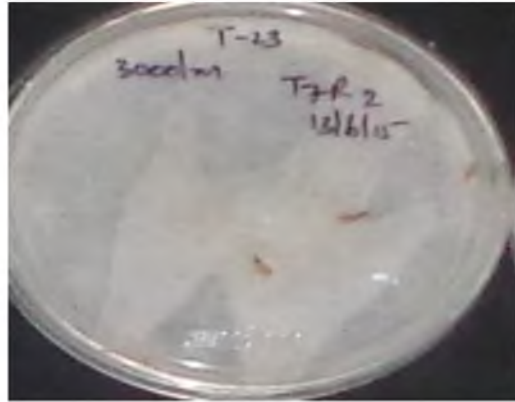


Fig. 6: Susceptibility of *M. domestica* larva on filter paper



Fig. 7: Susceptibility of *M. domestica* larvae in rearing medium



Fig. 8: Susceptibility of *M. domestica* larvae in poultry manure

in a completely random design. Plastic containers were examined at 1, 2, 3, 4, 5 and 10 days after treatment (DAT) and mortality was recorded based on death of larvae and adult fly emergence. Cadavers were examined for signs of nematode infection (i.e., coloration) (Woodring and Kaya, 1988).

3.9 Reproduction of EPN on *M. domestica*

Third instar larvae of housefly were exposed individually to IJs in petri dish (5 cm diam). Each petri dish was lined with filter papers and 3000 IJs were added in 1 ml of distilled water. The petri dishes were sealed with parafilm and incubated in the dark at $25 \pm 2^\circ \text{C}$ until larval death. The cadavers were rinsed in sterile distilled water to remove nematodes from their surface body. Then cadavers were incubated at $25 \pm 2^\circ \text{C}$ for 7 and 10 days for *Steinernema* and *Heterorhabditus*, respectively. The IJs that emerged from cadavers were collected over 2 days. There were 4 replicates and petri plates were placed in a completely random design.

3.10 Statistical analysis

Probit analysis was used to calculate LC_{50} and LC_{90} values (numbers of IJs/individual causing 50% and 90% mortality) and to calculate the respective 95% confidence intervals. The arcsine transformation was used to normalize the percentage data before an ANOVA was conducted. Analysis was undertaken on the transformed data and back transformed data only was presented. Nematode species, time, and their interactive effects on per cent survival and nematode species, dose and time, and their interactive effects on mortality percentage of different developmental stages of house fly was done by using PROC GLM (SAS version 9.3; SAS institute). When ANOVA was

significant, comparisons of relevant means were made using the Turkey's significance test values at the 5% level of significance.

3.11 Evaluation of biopesticides against fly control

3.11.1 Rearing of house fly

Adult flies were provided with milk and granulated sucrose. Then flies were transferred to artificial diet to lay eggs (Wheat bran meal with wheat bran+ milk powder+ egg yolk (7:3:1) (Fig. 9). Eggs were collected and then transferred to fresh artificial diet. Larvae were reared on artificial diet. The adults on emergence were fed with diluted honey solution. Bioassays were conducted on different developmental stages of housefly obtained through this rearing method.

3.11.2 Source of essential oils

Sweet basil (*Ocimum basilicum*) and cumin seed (*Cuminum cyminum*) were received from section of Medical and Veterinary pests, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. Curcumin (*Curcuma longa*) was obtained from Sigma Aldrich, Bangalore. *Azadirachta indica* -Neemazol[®] -F 5%, Parys Bio, Chennai, Tamil Nadu). Curcumin and neem was diluted with acetone and methanol respectively to different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm for various bioassays.

3.12 Bioassay

The bioassay was undertaken to find the efficiency of sweet basil (*Ocimum basilicum*), cumin seed (*Cuminum cyminum*), neem (*Azadirachta indica*) and curcumin (*Curcuma longa*) at different concentrations on houseflies.



Fig. 9: Rearing of *M. domestica*

The methodology of all the bioassays was done as per Kumar *et al.* (2011, 2012a) with slight modifications.

3.12.1 Fumigation assay

Fumigation assays were performed in a 50ml plastic container (66.66 cm^3). Filter paper strips of size 2x2 cm were attached to the lid of the container, later these strips were impregnated with different concentration of oils. The eggs, adults, larvae and pupae were placed at the bottom surface of the container.

3.12.2 Fumigation -adulticidal bioassay

For adulticidal bioassay, five lab reared adults were placed at the bottom of 50ml plastic container after anaesthetising the flies with petroleum ether for 30sec and they were provided with the diluted honey solution in a cotton ball. Different concentrations of oils (sweet basil and cumin seed) taken were 0.5, 1, 2, 3, 4 and $5 \mu\text{l}/66.66 \text{ cm}^3$ ($1 \mu\text{l}$ of basil=0.92mg and $1 \mu\text{l}$ of cumin seed=0.91mg) of air were loaded on the filter paper strips of 2x2 cm. Three replicates of each oil treatment were performed while control was treated with acetone. Curcumin and neem were inoculated with $50 \mu\text{l}$ of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97ppm. The controls were inoculated with the acetone and methanol, respectively. Treated filter paper was air dried for 2 min before introduction of adult for complete evaporation of acetone and methanol. The mouth of the container was tightly wrapped with parafilm (Fig. 10). The mortality of the flies in the container was recorded at 2h, 3h and 24h, respectively. All bioassays were performed at room temperature ($27 \pm 2^\circ\text{C}$).

3.12.3 Fumigation -larvicidal bioassay

The larvicidal bioassay was done in five lab reared larvae (third instars) which were placed at the bottom of plastic container. Different concentrations of oils (sweet basil and cumin seed) taken were 2, 4, 6, 8 and 10 μ l/66.66 cm³ (1 μ l of basil=0.92mg and 1 μ l of cumin seed=0.91mg) of air and loaded on the filter paper strips of 2x2 cm. Three replicates of each oil treatment were performed while control was treated with acetone. Curcumin and neem were inoculated with 50 μ l of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were inoculated with the acetone and methanol, respectively. Treated filter paper was air dried for 2 min before introduction of larvae for complete evaporation of acetone and methanol. The mouth of the container was tightly wrapped with parafilm (Fig. 11). The mortality of the larvae in the container was recorded at 2h, 3h, 24h, 48h and 72h, respectively. All bioassays were performed at room temperature (27 \pm 2⁰C).

3.13.4 Fumigation -pupicidal bioassay

Pupicidal bioassays were performed with five housefly pupae (2 day old) obtained through rearing of housefly under optimum condition. Different concentrations of oils (sweet basil and cumin seed) taken were 2, 4, 6, 8 and 10 μ l/66.66 cm³ (1 μ l of basil=0.92mg and 1 μ l of cumin seed=0.91mg) of air were loaded on the filter paper stripes of 2x2 cm were assessed in fumigation assay. Each treatment was replicated thrice while control was acetone treated. Curcumin and neem were inoculated with 50 μ l of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were inoculated with the acetone and methanol, respectively. Treated

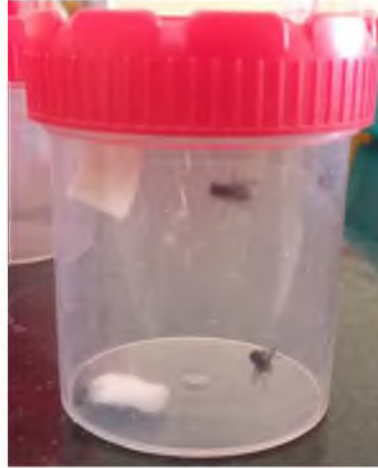


Fig. 10: Fumigant toxicity of *M. domestica* adults



Fig. 11: Fumigant toxicity of *M. domestica* larvae

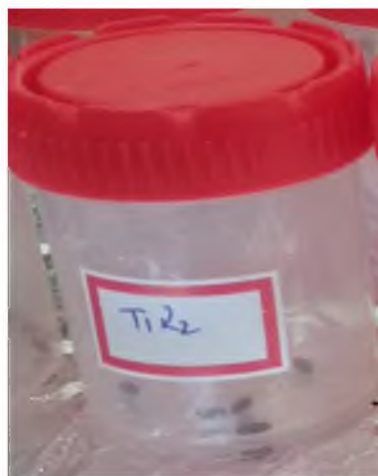


Fig. 12: Fumigant toxicity of *M. domestica* pupae

filter paper was air dried for 2 min before introduction of pupae for complete evaporation of acetone and methanol (Fig. 12). All bioassays were performed at room temperature ($27\pm 2^{\circ}\text{C}$). Observations were made at 7 days and activity of the oil was adjudged by calculation of percentage inhibition rate of adult emergence

$$\% \text{ IR (Percent inhibition rate)} = \frac{C_n - T_n}{C_n} \times 100$$

Where C_n is the number of newly emerged insects in the untreated (control) container and T_n is the number of insects in the treated container.

3.13.5 Fumigation -Ovicidal bioassay

Eggs were collected and then placed five per replicate on a moist filter paper in a container (66.66 cm^3). Different concentrations of oils (sweet basil and cumin seed) taken were 2, 4, 6, 8 and $10 \mu\text{l}/66.66 \text{ cm}^3$ ($1 \mu\text{l}$ of basil= 0.92mg and $1 \mu\text{l}$ of cumin seed= 0.91mg) of air were loaded on the filter paper strips of $2 \times 2 \text{ cm}$ were assessed in fumigation assay. Each treatment was replicated thrice while control was acetone treated. Curcumin and neem were inoculated with $50 \mu\text{l}$ of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97ppm. The controls were inoculated with the acetone and methanol, respectively. Treated filter paper was air dried for 2 min before introduction of eggs for complete evaporation of acetone and methanol. The mouth of the container was closed tightly and wrapped with parafilm tape. All bioassays were performed at room temperature ($27\pm 2^{\circ}\text{C}$). Observations were made at 24h and activity of the oil was adjudged by calculation of percentage inhibition rate of larval emergence.

$$\% \text{ IR (Percent inhibition rate)} = \frac{C_n - T_n}{C_n} \times 100$$

Where C_n is the number of newly emerged larva in the untreated (control) container and T_n is the number of larva in the treated container.

3.14 Contact toxicity bioassay

3.14.1 Topical toxicity bioassay:

3.14.1.1 Topical -adulticidal bioassay

The adult flies were collected from the rearing cage and were introduced in to small containers (66.66 cm^3) @ 5 insects after anaesthetizing flies by using petroleum ether for 30sec and they were provided with the diluted honey solution in a cotton ball. The flies were topically applied with $1 \mu\text{l}$ of different concentrations of essential oils (sweet basil and cumin seed) at 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and $0.1 \mu\text{l}/\text{fly}$ ($1 \mu\text{l}$ of basil=0.92mg and $1 \mu\text{l}$ of cumin seed=0.91mg) dissolved in acetone. Each treatment was replicated thrice while control was acetone treated. Curcumin and neem were applied with $1 \mu\text{l}$ of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were applied with the acetone and methanol, respectively. The toxicants were placed over the thorax. The mortality of the flies was observed after 2, 3 and 24 h. All bioassays were performed at room temperature ($27 \pm 2^\circ\text{C}$).

3.14.1.2 Topical- larvicidal bioassay

The housefly larvae were collected from the rearing cage and were introduced in to small containers (66.66 cm^3) @ 5 larvae. The larvae were topically applied with $1 \mu\text{l}$ of different concentrations of essential oils (sweet basil and cumin seed) at 0.1, 0.2, 0.3, 0.4 and $0.5 \mu\text{l}/\text{fly}$ ($1 \mu\text{l}$ of basil=0.92mg and $1 \mu\text{l}$ of cumin seed=0.91mg) dissolved in

acetone. The toxicants were placed over the skin of larva at the cephalopharyngeal region. Each treatment was replicated thrice while control was acetone treated. Curcumin and neem were applied with 1µl of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were applied with the acetone and methanol, respectively. The mortality of the larva was observed after 2, 3, 24 and 48 h. All bioassays were performed at room temperature ($27\pm 2^{\circ}\text{C}$).

3.14.1.3 Topical bioassay- pupae

The housefly pupae were collected from the rearing medium and were introduced in to small containers (66.66 cm^3) @ 5 pupae. The pupae were topically applied with 1µl of different concentrations of essential oils (sweet basil and cumin seed) at 0.05, 0.1, 0.5 and 1 µl/fly (1 µl of basil=0.92mg and 1 µl of cumin seed=0.91mg) dissolved in acetone. The toxicants were placed on the pupae. The treated pupae were transferred into the container. Each treatment was replicated thrice while control was acetone treated. Curcumin and neem were applied with 1µl of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97ppm. The controls were applied with the acetone and methanol, respectively (Fig. 13). Observations were made at 7 days and activity of the oil was adjudged by calculation of percentage inhibition rate of adult emergence.

$$\% \text{ IR (Percent inhibition rate)} = \frac{C_n - T_n}{C_n} \times 100$$

Where C_n is the number of newly emerged insects in the untreated (control) container and T_n is the number of insects in the treated container.

3.15 Feed toxicity bioassay

For each larvicidal bioassay, about one gram of artificial diet (wheat bran: milk powder at 8:2 ration and mixed with water) was placed in container. The diet was inoculated with the different concentrations of essential oils (sweet basil and cumin seed) at 5, 10, 15 and 20 $\mu\text{l/g}$ (1 μl of basil=0.92mg and 1 μl of cumin seed=0.91mg) of food and mixed thoroughly. Larvae were introduced in to small containers (66.66 cm^3) @ 5 larvae. Each oil treatment was replicated thrice. Control diet was inoculated with acetone. Curcumin and neem were inoculated with 50 μl of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were inoculated with the acetone and methanol, respectively. Treated diet was air dried for 5 min before introduction of larvae for complete evaporation of acetone and methanol (Fig. 14). The mortality of the flies was observed after 2, 3, 24 and 48 h. All bioassays were performed at room temperature ($27\pm 2^{\circ}\text{C}$).

3.16 Residual film coat bioassay

3.16.1 Residual film coat adulticidal bioassay

For residual film coat bioassay a glass container (24.20 cm^3) was coated with different concentrations of essential oils (sweet basil and cumin seed) at 2, 2.5, 3, 3.5 and 4 μl (1 μl of basil=0.92mg and 1 μl of cumin seed=0.91mg) dissolved in 500 μl of acetone/ 24.20 cm^3 . The containers were rolled carefully for proper and even distribution of oils. Each oil treatment was replicated thrice. Control was inoculated with acetone. Curcumin and neem were inoculated with 500 μl of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were applied with the acetone and methanol, respectively. The treated container was air dried for 5 min

before introduction of adult for complete evaporation of acetone and methanol. The adult flies were collected from the rearing cage and were introduced in to container @ 5 insects after anaesthetizing flies by using petroleum ether for 30sec and they were provided with the diluted honey solution in a cotton ball (Fig. 15). The mortality of the flies was observed after 2, 3 and 24 h. All bioassays were performed at room temperature ($27\pm 2^{\circ}\text{C}$).

3.16.2 Residual film coat larvicidal bioassay

For residual film coat bioassay a glass container (24.20 cm^3) was coated with different concentrations of essential oils (sweet basil and cumin seed) at 2, 2.5, 3, 3.5 and 4 μl (1 μl of basil=0.92mg and 1 μl of cumin seed=0.91mg) dissolved in 500 μl of acetone/ 24.20 cm^3 . The containers were rolled carefully for proper and even distribution of oils. Each oil treatment was replicated thrice. Control tubes were coated with acetone. Curcumin and neem were inoculated with 500 μl of different concentrations of 50000, 25000, 12500, 6250, 3125, 1563, 781, 390, 195 and 97 ppm. The controls were coated with the acetone and methanol, respectively. Treated container was air dried for 5 min before introduction of larvae for complete evaporation of acetone and methanol. The third instars were collected from the rearing medium and were introduced into container @ 5 larvae per treatment (Fig. 16). The mortality of the larvae was observed after 2, 3, 24 and 48 h. All bioassays were performed at room temperature ($27\pm 2^{\circ}\text{C}$).

3.17 Repellency on housefly larvae

Repellency on housefly larvae done as per Kerdchoechuen *et al.*, 2010 with slight modification. The housefly larvae were collected from the rearing cage and were kept

starved for 4-5 hours. 1g of artificial diet was taken and placed on two corner sides of a petridish (9cm). Different concentrations of essential oils (sweet basil and cumin seed) at 10 and 25 $\mu\text{l/g}$ (1 μl of basil=0.92mg and 1 μl of cumin seed=0.91mg) of artificial diet which was placed on the one side of the petridish and similarly artificial diet which was placed on the other quadrant of the petridish was untreated (control). Curcumin was added at the rate of 100 μl in different concentrations of 50000, 25000, 12500 and 6250 ppm. The controls were treated with the acetone. Neem was applied at the rate of 50 and 100 μl with 50000 ppm. Starved larvae were introduced in to the petridish @ 5 larvae per petridish at middle of the plate. Movement of the each larva in the petridish were observed for 5, 10, 20, 30 minutes and 1h (Fig.17).

Percentage repellency (PR) was calculated by the formula:

$$\text{PR} = \left[\frac{(\text{Nc} - \text{Nt})}{\text{Tn}} \right] 100.$$

Tn-Total number of larvae introduced at the middle of the plate

Nc- The number of larvae present at the control half (Nc)

Nt- The number of larvae present at the treated half (Nt)

3.18 Ovipositional deterrent bioassay

About 50 housefly adults of both sexes aged 5days old were collected randomly from the rearing cage and were introduced into each new cage. In each cage the flies were provided with diluted sugar solution as food source and with water. Two petriplates (9cm) containing artificial diet, one with the 200 μl of essential oils (sweet basil, cumin seed, neem and curcumin) (1 μl of basil=0.92mg and 1 μl of cumin seed=0.91mg) another petriplate without any essential oil as control were kept in cages with a



Fig. 13: Topical bioassay of *M. domestica* pupae

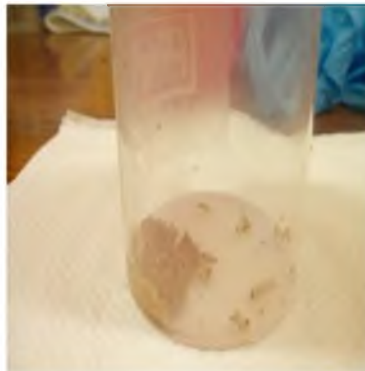


Fig. 14: Feed toxicity of *M. domestica* larvae



Fig. 15: Residual film coat toxicity of *M. domestica* adults



Fig. 16: Residual film coat toxicity of *M. domestica* larvae

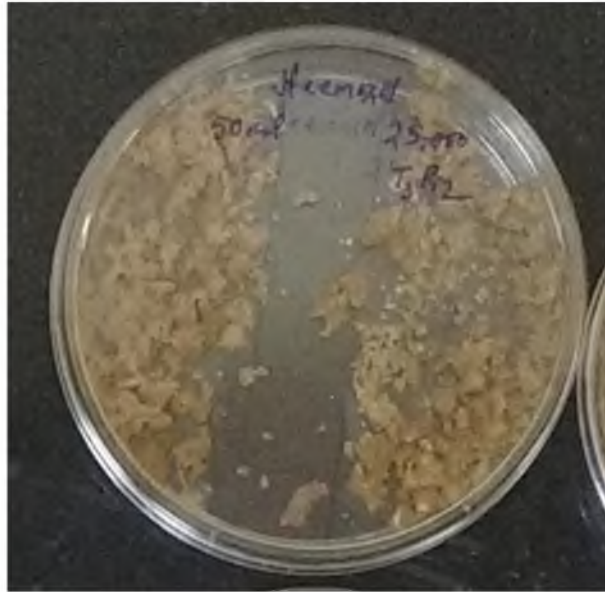


Fig. 17: Repellency of botanicals on *M. domestica* larvae



Fig. 18: Ovipositional deterrent bioassay

distance between each plate. Separate cages were used for each essential oil. The egg laying was observed after 12 h (Fig. 18). The eggs laid into each box were collected separately after 24 h and were counted. The percentage of effective repellency for each essential oil was calculated using the following formula (Soonwera, 2015).

$$ER\% = \frac{NC - NT}{NC} \times 100$$

Where ER = effective repellency

NC = the total number of eggs in the control

NT = the total number of eggs in each test

3.19 Statistical analysis

Data obtained from each dose and time response bioassay were subjected to regression analysis by probit to generate values for LC_{50} , LC_{99} by SPSS software. The effect of varying doses vs. different exposure periods on larval mortality and pupal inhibition rate were analysed with two-way analysis of variance.

3.20 Evaluation of new modes of application with semiochemicals

In the present study a new mode of application of insecticides was evaluated by Semiochemicals- “Attraction and kill” strategies. Combining an attractant (semiochemicals) with an insecticide for slow release for control of flies by incorporating in a gel matrix.

3.20.1 Source of chemicals

Sugar –normal food grade, water-distilled water and gel matrix forming substance received from section of Medical and Veterinary pests, ICAR-National Bureau of

Agricultural Insect Resources (NBAIR) Bengaluru; Z-9-tricosene and α -terpeniol obtained from Sigma Aldrich, Bengaluru; imidacloprid (Victor[®] 17.8% from Mayuri Agencies, Ghatanji Yavatmal KarimNagar).

3.21 Preparation of gel matrix and dissipation pattern of gel

Gel matrix was prepared by adding 500mg of gel forming substance (provided by NBAIR, Bangalore) and 2500 mg sugar to 50 ml of water. After preparation of 50 ml of gel it was equally divided into four quadrants of 12.5 g and taken in separate petri plate. The plates were weighed before and after putting the gel and the plates were kept at different temperature such as room temperature, 35⁰ C and 40⁰ C. Weight of the plates were taken 1, 2, 3, 4h and 24h to 7 days for the rate of dissipation.

3.22 Evaluation of different semiochemical baits in laboratory:

Different types of semiochemical target baits were prepared by incorporating different attractants and imidacloprid as insecticide are given below.

50ml water+2.5g sugar+50 μ l of Z-9-tricosene+50 μ l imidacloprid+0.5g gel matrix

50ml water+2.5g sugar+50 μ l imidacloprid+0.5g gel matrix

50ml water+2.5g sugar+50 μ l of α -terpeniol+50 μ l imidacloprid+0.5g gel matrix

50ml water+2.5g sugar+1g gel matrix

After preparation of target baits of 50 ml capacity it was equally divided into two petri plates and kept in a cage containing 50 adult flies which were randomly collected from the rearing cages and observed for the mortality of flies.

3.23 For field assay (in waste disposal management unit and poultry farm) the following target baits were prepared.

100ml water+5g sugar+100 μ l of Z-9-tricosene+100 μ l imidacloprid+1g gel matrix

100ml water+5g sugar+100 μ l imidacloprid+1g gel matrix

100ml water+5g sugar+20g quick bite (imidacloprid granules) +1g gel matrix

100ml water+5g sugar+100 μ l of α -terpeniol+100 μ l imidacloprid+1g gel matrix

These toxic baits were spread on butter sheets. Each toxic bait was replicated thrice and the observations on mortality of flies taken were at 1h, 3h and 24h-72h. The number of flies dead in each bait were counted.

3.24 Evaluation of agripower diatomite (diatomaceous earth) for control of flies

The diatomaceous earth was procured from Sigma Aldrich, Bangalore. Two varieties were used *viz.* Diatomaceous earth powder, suitable for most filtrations (DE, calcined) and Diatomaceous earth powder non-washed powder (DE, Diatomaceous silica, Kieselguhr).

3.24.1 Diatomaceous earth application to adult feed

The adult feed was prepared by mixing 2g of sugar in 10ml of water. This 10 ml solution was mixed with 2g and 4g of different grades of diatomaceous earth where as the control contained only sugar solution. The solution was divided into three parts and taken on a cotton balls and put into the petriplates of 9cm, later 10 flies were released in each plate and observed for the mortality of the flies daily.

3.24.2 Diatomaceous earth application to artificial diet of larvae

About 10g larval diet was prepared by mixing wheat bran and milk powder in the ratio of 8:2. 10g of food was mixed with 2g and 4g of different grades of diatomaceous earth whereas the control did not receive any DE. The treated diet was divided into three parts and put into three different petri plates and 10 third instar larvae was taken in each petriplate and observed for the pupation and emergence of adult flies.

3.24.3 Diatomaceous earth application to poultry manure

Manure samples (200 g of manure in plastic containers) were treated at the concentrations of 2, 4, 8, 10, 20, 40, 60, 80 and 100% of different grades of DE. The powder was mixed properly in manure to ensure homogeneity and 25 third instar larvae of *M. domestica* on the top of treated manure using a fine-tip paint brush and reared until adult emergence. The control was not treated with the DE powder. Containers were placed at room temperature and three replicates of each treatment were performed (Shiell, 2015).

Results



IV. RESULTS

4.1 Prevalence and diversity of fly infestation in poultry farms

The seasonal prevalence of different flies was studied in eleven different poultry farms in Bangalore districts as detailed in Table 1. The flies were collected with sweep net during rainy, winter and summer seasons.

A total of six different flies were found to occur viz. *Musca domestica*, *Chrysomya megacephala*, *Hydrotaea aenescens*, *Hydrotaea capensis*, *Hermetia illucens* and *Sarcophaga ruficornis*.

Seasonal prevalence of flies

In the rainy season all six variety of flies were prevalent, where as in winter and summer only *M. domestica*, *C. megacephala* and *Hermetia illucens* were prevalent (Table. 5).

Table. 5: Overall seasonal prevalence of flies in poultry farms of Bangalore district

Species	Rainy	Winter	Summer
<i>M. domestica</i>	+	+	+
<i>C. megacephala</i>	+	+	+
<i>Hy. aenescens</i>	+	-	-
<i>Hy. capensis</i>	+	-	-
<i>He. illucens</i>	+	+	+
<i>Sarcophaga ruficornis</i>	+	-	-

+ Prevalent, - Not prevalent

Farm wise seasonal prevalence of flies

Farm wise prevalence of different flies given in (Table. 6). In Pragathi Poultry Farm, Nelmangala, Bangalore rural, *M. domestica*, *C. megacephala* and *S. ruficornis* were prevalent in rainy season, whereas in winter *M. domestica* and *C. megacephala* were prevalent and in summer only *M. domestica* was prevalent.

In Venkateshwara Breeding Farm, Kannur, Bangalore East, in rainy season, *M. domestica* and *C. megacephala* and *H. aenescens* were prevalent whereas in winter *M. domestica*, *C. megacephala* were prevalent and in summer *M. domestica* and *C. megacephala* were prevalent.

In Monarca Hatcheries, Honnenahalli, Bangalore North, in rainy season *M. domestica*, *C. megacephala* were prevalent, whereas in winter *M. domestica*, *C. megacephala* were prevalent and in summer only *M. domestica* was prevalent.

In CPF Poultry Farm, Mandur, Bangalore East, in rainy season *M. domestica*, *C. megacephala* and *H. capensis* were prevalent, whereas in winter *M. domestica*, *C. megacephala* were prevalent and in summer *M. domestica*, *C. megacephala* were prevalent.

In Davangere poultry farm, Dhavangere *M. domestica* and *He. illuscense* were found to be prevalent in all the seasons.

In Rajeshwari Poultry Farm (Hoskote, Bangalore rural), Thangaraju Poultry Farm (Hancharahalli, Bangalore East), State Poultry Farm (Hessarghatta, Bangalore rural), Jagadeesh Poultry Farm (Hessarghatta, Bangalore rural), KVAFSU Poultry Farm

(Hebbal, Bangalore) and Bannerghatta Poultry Farm (Bangalore rural) only *M. domestica* was prevalent in all the seasons.

Prevalence of flies depending on the system of rearing

In deep litter system of rearing, only *M. domestica* was found to be prevalent, whereas in the case of cage system of rearing, all six different flies species were found to be prevalent (Table. 6) viz., *M. domestica*, *C. megacephala*, *Hy. aenescens*, *Hy. capensis*, *He. illucens* and *S. ruficornis*.

Prevalence of flies in different farms and seasons

The seasonal prevalence of flies in different farms is detailed in Table. 6.

Musca domestica was found to be prevalent in all the farms and in all the seasons. It was found to occur in all systems of rearing (cage and deep litter). It was the most abundant and most common fly species prevalent in poultry farms.

Chrysomya megacephala was found in only four farms in the present study, viz., Pragathi Poultry Farm (Nelamangala, Bangalore rural), Venkateshwara Preeding Farm (Kannur, Bangalore East), Monarca Hatcheries (Honnenahalli, Bangalore North) and CPF Poultry Farm (Mandur, Bangalore East). Among these farms, it was prevalent in all the seasons Venkateshwara Preeding Farm in Kannur (Bangalore East), Monarca Hatcheries and CPF Poultry Farm, Mandur. In the case of Pragathi Poultry Farm (Nelamangala, Bangalore rural) and Monarca Hatcheries (Honnenahalli, Bangalore North), the species was prevalent in rainy and winter season but was not found during the summer season. In the remaining farms this species was not found. It was found in cage system of rearing and was not found in deep litter system of rearing.

Table. 6: Farm wise prevalence of flies in poultry farms of Bangalore district

Places of collection	Type of rearing	Rainy	Winter	Summer
Pragathi poultry farm, Nelmangala, Bangalore rural	Cage	<i>M. domestica</i> , <i>C. megacephala</i> , <i>S. ruficornis</i>	<i>M. domestica</i> , <i>C. megacephala</i>	<i>M. domestica</i>
Venkateshwara breeding farm, Kannur, Bangalore East	Cage	<i>M. domestica</i> , <i>C. megacephala</i> , <i>Hy. aenescens</i>	<i>M. domestica</i> , <i>C. megacephala</i>	<i>M. domestica</i> , <i>C. megacephala</i>
Monarca hatcheries, Honnenahalli, Bangalore North	Cage	<i>M. domestica</i> , <i>C. megacephala</i> ,	<i>M. domestica</i> , <i>C. megacephala</i>	<i>M. domestica</i> ,
Rajeshwari poultry farm, Hoskote, Bangalore rural	Cage	<i>M. domestica</i>	<i>M. domestica</i>	<i>M. domestica</i>
Thangaraju poultry farm, Hancharahalli, Bangalore East	Deep litter	<i>M. domestica</i>	<i>M. domestica</i>	<i>M. domestica</i>
CPF poultry farm, Mandur, Bangalore East	Cage	<i>M. domestica</i> , <i>C. megacephala</i> , <i>Hy. capensis</i>	<i>M. domestica</i> , <i>C. megacephala</i>	<i>M. domestica</i> , <i>C. megacephala</i>
State poultry farm, Hessarghatta, Bangalore rural	Deep litter	<i>M. domestica</i>	<i>M. domestica</i>	<i>M. domestica</i>
Jagadeesh poultry farm, Hessarghatta, Bangalore rural	Cage	<i>M. domestica</i>	<i>M. domestica</i>	<i>M. domestica</i>
KVAFSU poultry farm, Hebbal, Bangalore	Cage, deep litter	<i>M. domestica</i>	<i>M. domestica</i>	<i>M. domestica</i>
Bannerghatta poultry farm, Bangalore rural	Cage	<i>M. domestica</i>	<i>M. domestica</i>	<i>M. domestica</i>
Davangerepoultry farm, Dhavangere	Cage	<i>M. domestica</i> , <i>He. illuscense</i>	<i>M. domestica</i> , <i>He. illuscense</i>	<i>M. domestica</i> , <i>He. illuscense</i>

Hydrotaea aenescens was found to be prevalent in Venkateshwara Breeding Farm, Kannur, Bangalore East during rainy season only. It was not found in other farms and in other seasons. It was found in the cage system of rearing and was not recorded in the deep system of rearing.

Hydrotaea capensis was found to be prevalent in CPF Poultry Farm, Mandur, Bangalore East, during rainy season only. It was not found in other farms and in other seasons. It was observed in the cage system of rearing and was not found in the deep litter system of rearing.

Hermetia illucens was found to be prevalent in Davangere Poultry Farm, Dhavangere in, all the seasons. It was not found in other farms. It occurred in the cage system of rearing and not in the deep litter system of rearing.

Sarcophaga ruficornis was found to be prevalent in Pragathi Poultry Farm, Nelmangala, during rainy season. It was not found in other farms and in other seasons. It was observed in the cage system of rearing and was not recorded in the deep litter system of rearing.

4.2 Morphological identification of flies

The characters for morphological identification of fly included length of fly, eyes, antennae, wing pattern, and colour of flies, thorax and abdominal pattern.

Basic morphological features of larva used for identification purpose: length of larvae, integument, spines, cephalopharyngeal skeleton, respiratory structures such as anterior and posterior spiracles.

Morphological features of different developmental stages of *M. domestica*

Egg (Fig. 19 and 20): Eggs were whitish measuring about 1.28mm long and oval in shape. Along the concave dorsal side two distinct rib-like hatching pleats with thickenings running parallel were present.

I instar larva (Fig. 20, 21, 28, 38, 43): The hatched larva was glistening white in colour and about 1.7mm long. The four anterior segments were bare; from the fifth to twelfth segment and there was a transverse, fusiform, swollen area provided with spines ventrally, occupying the anterior third of the segment. At the posterior part of sixth and seventh segments, there was one row of spines ventrally, pressed against the spinose area of the following segment. These spines increased to three rows on segment 8-12. There were five short rows of spines dorsal to the anal opening. The spines were of various shapes-triangular, dome shaped and wedge shaped. The posterior spiracles were present as two dots.

II instar larva (Fig. 23, 30, 34, 39, 45): They measured about 5.7mm in length. The anterior ends of segments two to five are provided with complete spinose rings. From sixth to twelfth segment, at the anterior end, there is a ventral transverse swollen spinose area which, continued laterally and dorsally by a single row of spines, forms a complete ring. The posterior ventral rows of spines in this stage were found on the fifth to the twelfth segment. Anterior spiracles with sixth to eight branches; the posterior each with two slits surrounded by a weak ring.

III instar larva (Fig. 25, 32, 36, 47): The larva measured 12mm in length. It was creamy white, the spinulation unpigmented and quite similar to that of second stage. The anterior

spiracles had five to seven branches, the posterior spiracles were D shaped with a median button each spiracle with three tortuous m shaped slits.

Adult (Fig. 49, 50, 57, 62): Thorax was greyish with four dark stripes. Propleural depression (in front of and somewhat below anterior spiracle) had some short black hairs. Abdomen was pale brownish at sides towards base. Male had holoptic eyes where the distance between two eyes was less where as in female dichoptic and the distance was more. Third segment of antenna had arista which was plumed on both sides. Females measured 5.6mm and males 5.18mm.

Wing (Fig. 71): The fourth longitudinal vein had an upward curve at about the middle of its course and joined the third longitudinal vein to form a closed apical cell. Length of female wing was 5.46mm and male 5.14mm.

Morphological features of different developmental stages of *C. megacephala*

I instar larva (Fig. 22, 29, 40, 44): Measured 3mm in length. The cephaloskeleton was well developed and showed a median chitinized process. Accessory spines were detectable around the straight labial sclerite.

II instar larva (Fig. 24, 31, 35, 41, 46): The second larval stage measured a length of 6.3mm. The labial sclerites were curved in second stage larva. The median process and accessory spines were lacking.

III instar larva (Fig. 26, 33, 37, 42, 47): The larva measured 16.58mm. The segments were armed with bands of spinules, the band on last segment was developed only

laterally and ventrally. The anterior spiracle had 13 branches. Peritreme was incomplete and heavily pigmented as a dark structure encircling three relatively straight spiracular openings (slits), with middle slits appearing slightly bent inward

Adult (Fig. 51, 52, 58, 63, 64, 65): It was bluish green in colour. Mesonotum had two short and narrow longitudinal black stripes anteriorly. Anterior thoracic spiracle was dark brown, genal dilation had orange ground color with orange seta. Wings were hyaline, slightly darkened at the base, legs were black. Head in the male had the eyes touching in the middle of the frons, facets of upper two third greatly enlarged and sharply demarcated from the small facets in lower third. In the female the eyes were separated by a broad frons and the upper facets were not strikingly enlarged and not demarcated from the lower ones. Face including buccae in both sexes was bright orange in colour. Length of female was 8.25mm and male was 8.14mm.

Wing (Fig. 72): Stem-vein with a row of setulae on upper posterior side; upper surface of lower lobe of squama hairy; upper squama white, partly dark margined; lower squama brown. Length of female wing was 7.6mm and male wing was 6.42mm.

Morphological features of *Hy. aenescens*

Adult (Fig. 53, 59, 60, 73): It measured 4.8mm in length. Palpus yellow or reddish yellow, hind tibia posteroventrally was without either bristles or long setaceous hairs, and anteroventrally with only two or three short bristles both mid and hind femora with some short, stout, erect spines on the ventral surface near the base. Frontal triangle elongate, extending the full length of the front up to the lunule, apically subtruncate or broadly rounded. Length of wing was 3.8mm.

Morphological features of *Hy. capensis*

Adult (Fig. 54, 66, 74): It measured 5.5mm in length. Parafrontal and upper third to half of the parafacial glabrous and polished black, highly shining. Shining bluish black or greenish black, with black palpi and white calypteres. Mesonotum was densely and uniformly covered with fine, erect hairs. Para frontal, long and ending acutely close to the lunule, the width of the triangle at the level of the cruciate interjfrontal bristles obviously wider than from the triangle to an eye opposite that point; both mid and hind femora with fine hairs on the ventral surface toward the base; hind tibia with two posterodorsal bristles, and ventrally with a row of three to five anteroventral bristles, the longest of which are only slightly longer than the diameter of the tibia, but without posteroventral bristles. Length of wing was 4.55mm.

Morphological features of *He. illucens*

Larva (Fig. 27): Was black in colour, elongate and flattened with exoskeleton firm and tough. It measured about 17mm in length. Head is small and narrower than the body. The body bore no other prominent features except body spines.

Adults (Fig. 55, 61, 67, 68): Adults were robust black in colour. The adult flies abdomen was slender consisting of 5 visible segments. The first abdominal segment has clear areas. Soldier flies also have two translucent “windows” located on the first abdominal segment. The head of adult flies was small and narrower than the body. Their eyes were broadly separated in both sexes. The antennae had a long terminal segment characterized by a elongated, flagellum. It was twice as long as the head. Length of adult fly was 13.5mm.

Wing (Fig. 75): Smoky black wings with microtrichia very dense and cover entire wing membrane. Length of wing was 10.2mm.

Morphological features of *Sarcophaga ruficornis*

Adult (Fig. 56, 69, 70): Measured about 12mm in length. Frons was equal to two fifth of an eye width. Frontal stripe black, parafrontalia yellowish above and silvery was in the face below. Antennae and palpi orange yellow. Third segment of antennae was twice that of second one. Thorax was grey in colour, propleura is bare. Three longitudinal stripes on the thorax were present with a brown prothorasic spiracle. Wings were hyaline with brown veins, squama was white and halteres were brown in colour. Abdomen was black with silvery checkered pattern. The colour of the male genital capsule was reddish brown in colour. Male genitalia revealed a penis with membranous bulbous portion in between theca and paraphallus, inner forceps densely covered with long hairs and ending in an acute point at the end, outer forcep somewhat oval, anterior paramere bent and pointed at end. Theca shorter than paraphalus and both were sclerotized.



Fig. 19: *M. domestica* egg (40X)

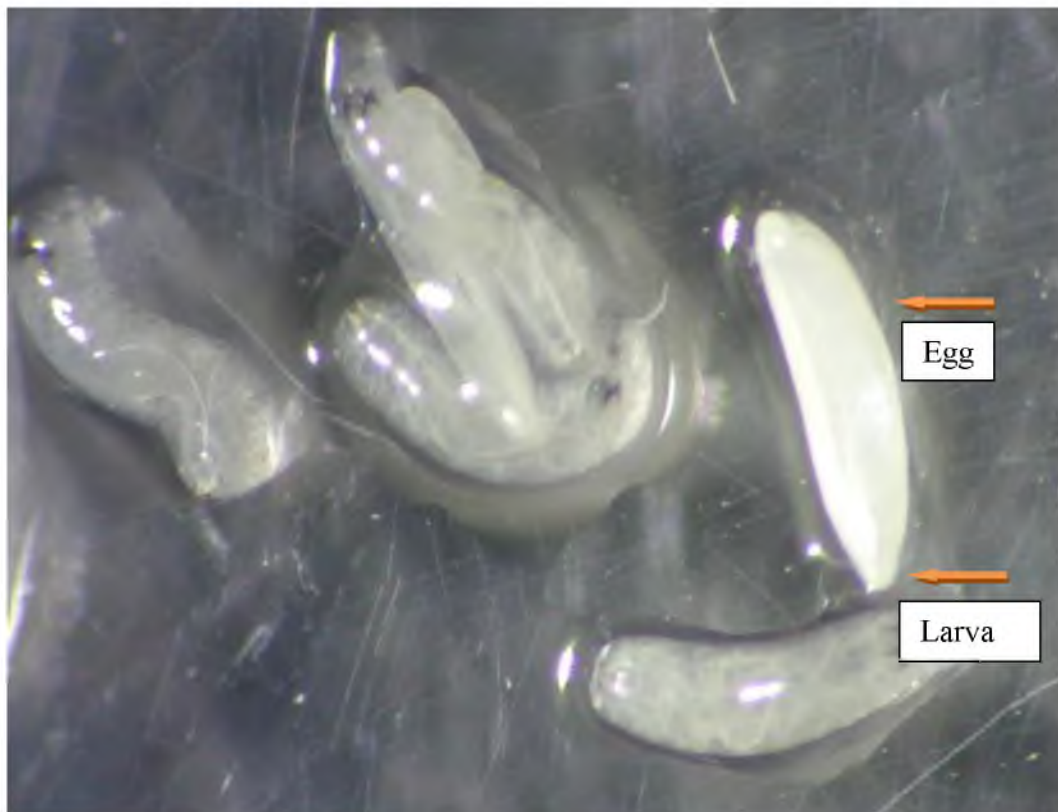


Fig. 19 and 20: Egg and I instar larva of *M. domestica*



Fig. 21: I instar larva of *M. domestica*



Fig. 22: I instar larva of *C. megacephala*



Fig. 23: II instar larva of *M. domestica*



Fig. 24: II instar larva of *C. megacephala*



Fig. 25: III instar larva of *M. domestica*



Fig. 26: III instar larva of *C. megacephala*



Fig. 27: III instar larva of *He. illucens*, head capsule



Fig. 28: Cephaloskeleton of I instar larva *M. domestica*

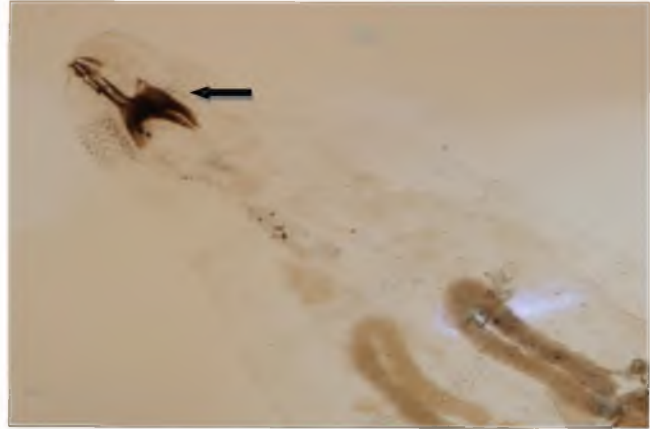


Fig. 29: Cephaloskeleton of I instar larva *C. megacephala* (40X)

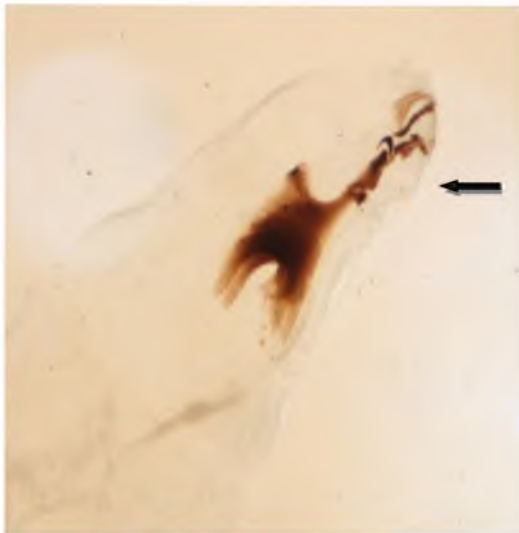


Fig. 30: Cephaloskeleton of II instar larva *M. domestica*

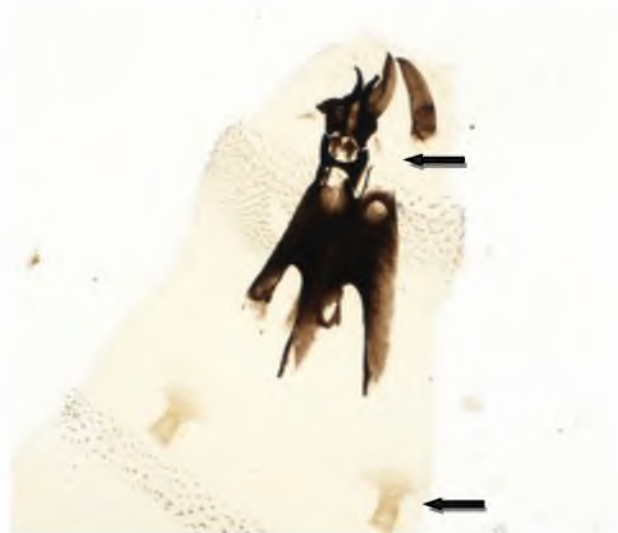


Fig. 31: Cephaloskeleton and anterior spiracles of II instar larva *C. megacephala* (40X)

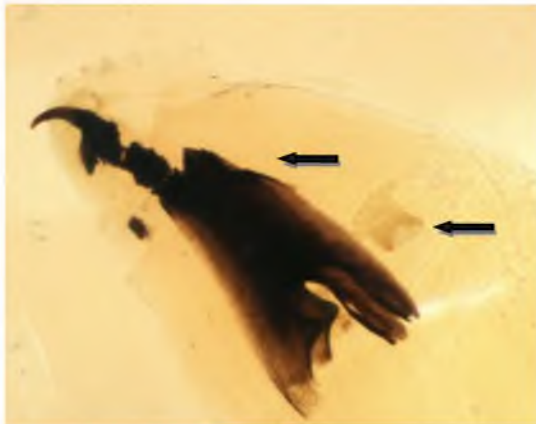


Fig. 32: Cephaloskeleton and anterior spiracles of III instar larva *M. domestica*

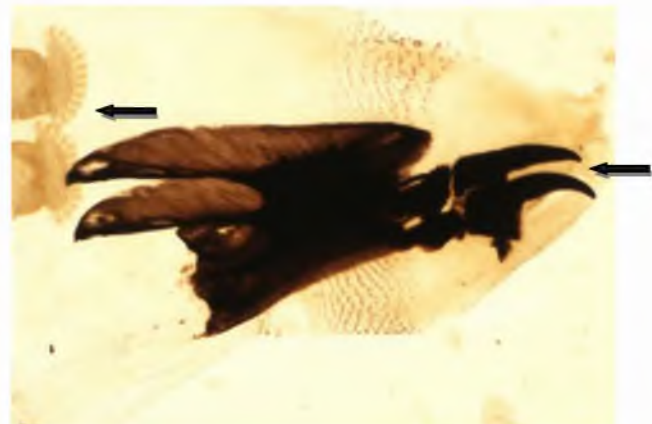


Fig. 33: Cephaloskeleton and anterior spiracles of III instar larva *C. megacephala* (40X)

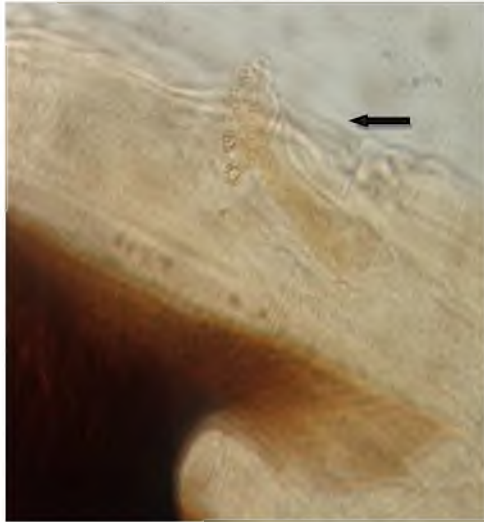


Fig. 34: Anterior spiracle of II instar larva *M. domestica*

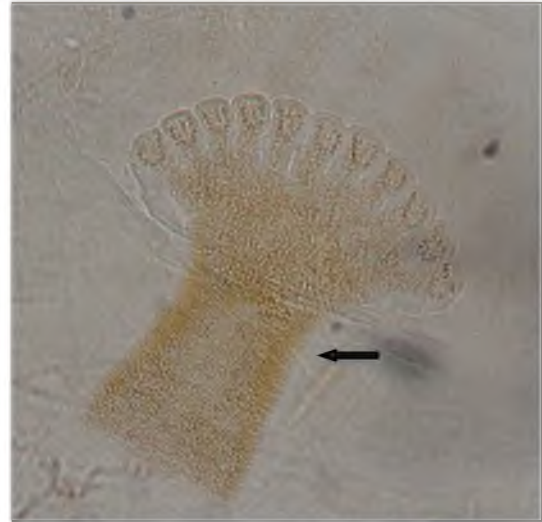


Fig. 35: Anterior spiracle of II instar larva *C. megacephala* (100X)



Fig. 36: Anterior spiracle of III instar larva *M. domestica*

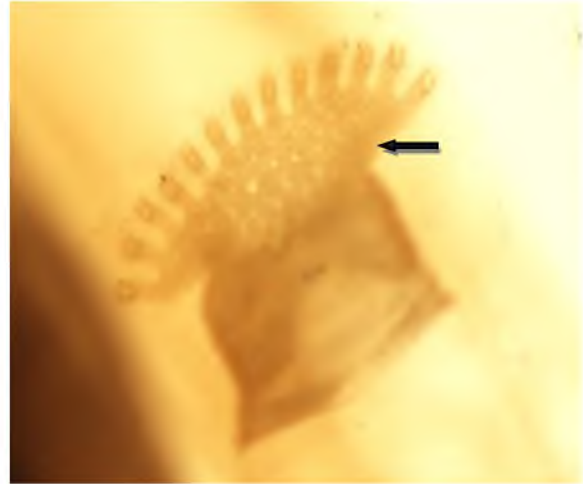


Fig. 37: Anterior spiracle of III instar larva *C. megacephala* (100X)



Fig. 38: Bands of spines of I instar larva *M. domestica*

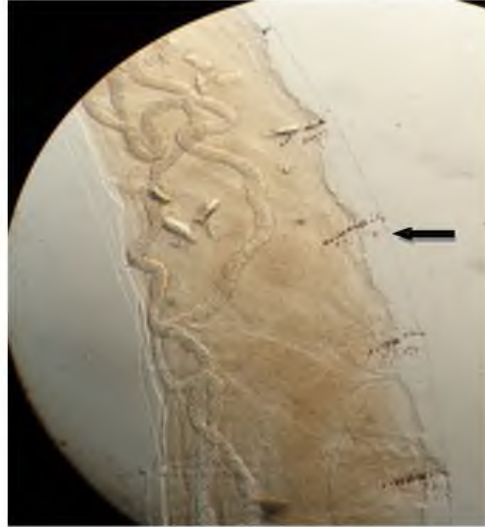


Fig. 39: Bands of spines of II instar larva *M. domestica* (40X)

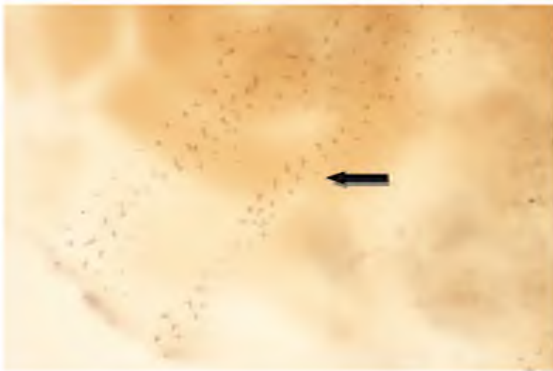


Fig. 40: Bands of spines of I instar larva *C. megacephala*



Fig. 41: Bands of spines of II instar larva *C. megacephala* (40X)

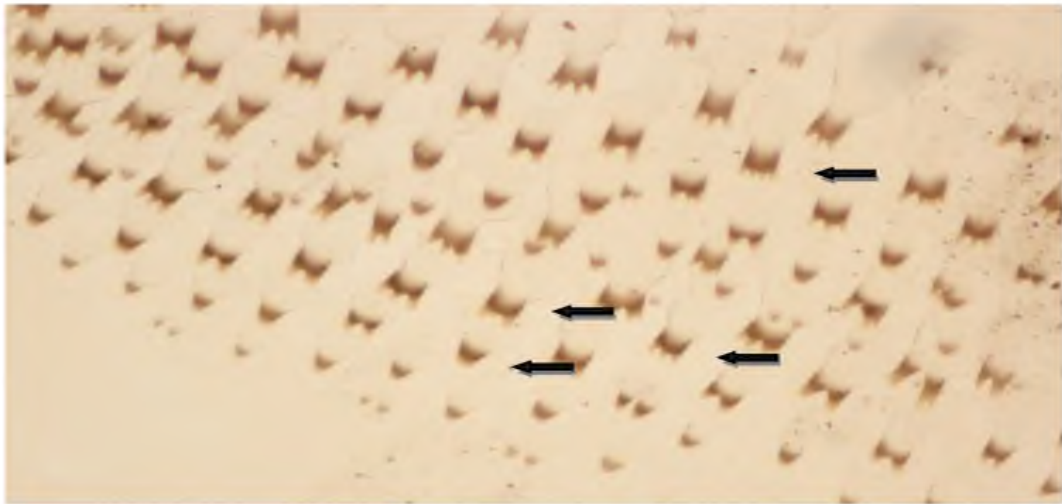


Fig. 42: Bands of spines of III instar larva *C. megacephala* (40X), division of spines

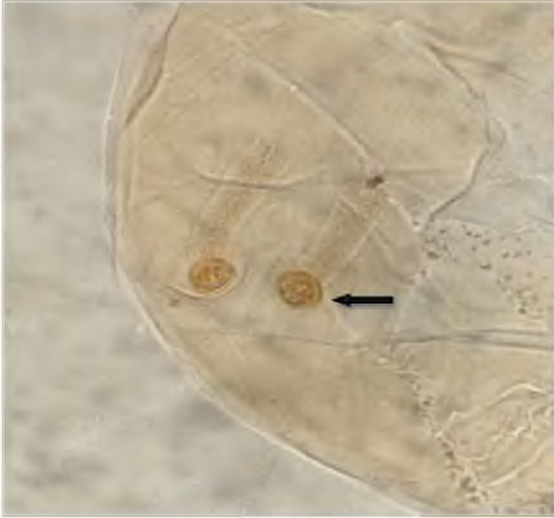


Fig. 43: Posterior spiracles of I instar larva *M. domestica*

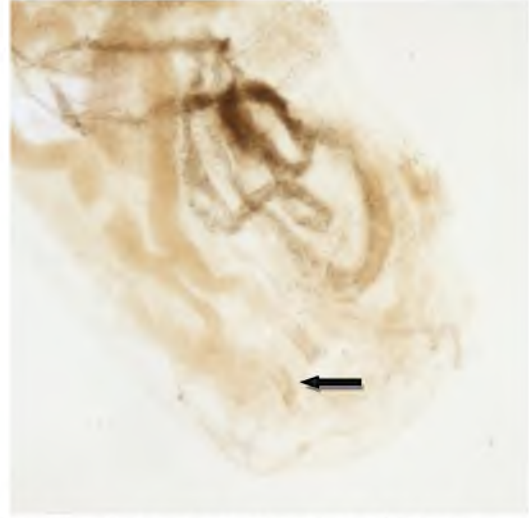


Fig. 44: Posterior spiracles of I instar larva *C. megacephala* (20X)



Fig. 45: Posterior spiracles of II instar larva *M. domestica*



Fig. 46: Posterior spiracles of II instar larva *C. megacephala* (40X)



Fig. 47: Posterior spiracles of III instar larva *M. domestica*

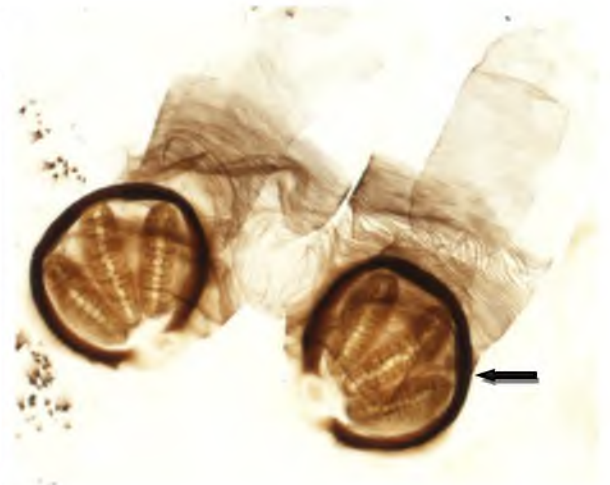


Fig. 48: Posterior spiracles of III instar larva *C. megacephala* (20X)



Fig. 49: Female head, anterior view-
dichoptic- *M. domestica*



Fig. 50: Male head, anterior view-
holoptic- *M. domestica*



Fig. 51: Female head, anterior view-
dichoptic - *C. megacephala*



Fig. 52: Male head, anterior view-
holoptic - *C. megacephala*
(enlargement of upper 2/3rd
facets of eye)

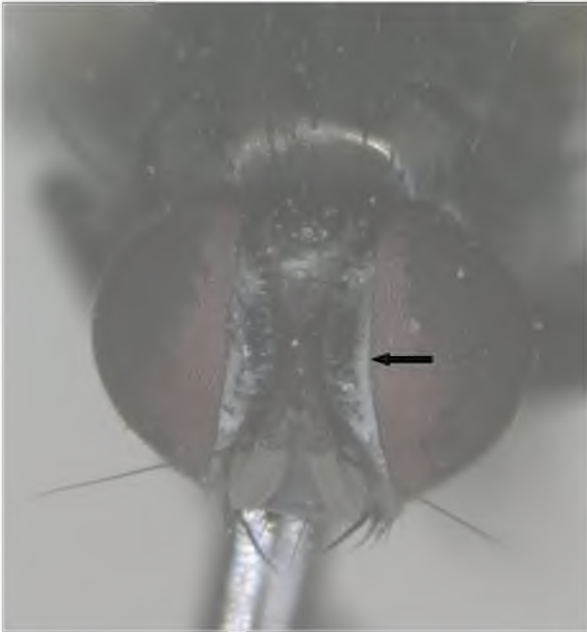


Fig. 53: Female head, anterior view-dichoptic - *Hy. aenescens*



Fig. 54: Female head, anterior view-dichoptic - *Hy. capensis*



Fig. 55: Female head, anterior view-dichoptic - *He. illucens*

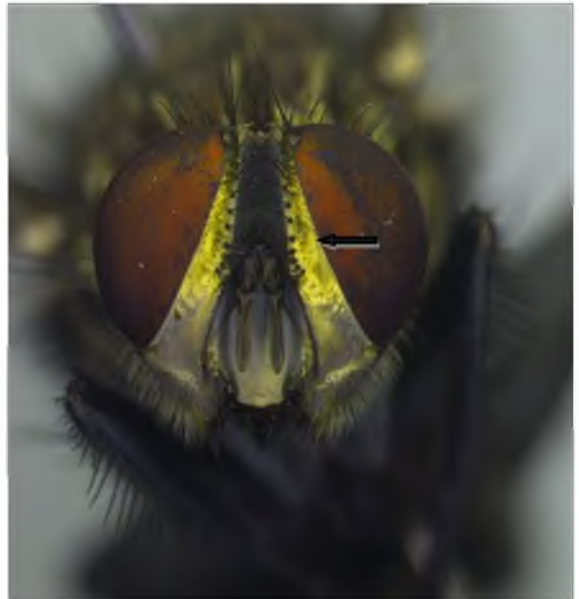


Fig. 56: Male head, anterior view-dichoptic - *S. ruficornis*



Fig. 57: Dorsal view of thorax of *M. domestica* showing 4 longitudinal stripes

Fig. 58: Dorsal view of thorax of *C. megacephala* showing 2 faint longitudinal stripes



Fig. 59: Dorsal view of thorax of *Hy. aenescens*



Fig. 60: Dorsal view of thorax of *Hy. aenescens*



Fig. 61: Dorsal view of thorax of *He. illucens*



Fig. 62: Lateral view of *M. domestica*, anterior thoracic spiracle

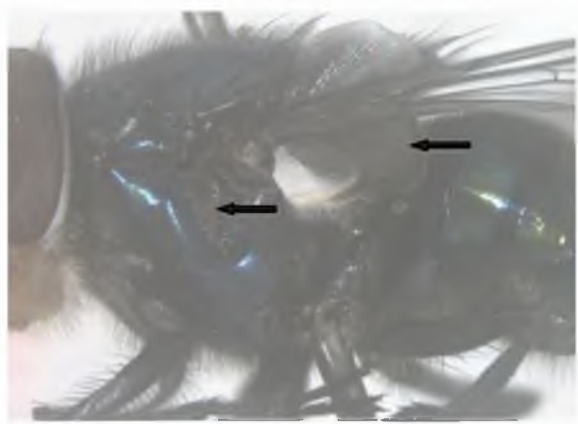


Fig. 63: Lateral view of *C. megacephala*, mesopleural bristles, brown color lower squama



Fig. 64: Lateral view of *C. megacephala*, brown anterior thoracic spiracle



Fig. 65: Antenna, arista plumed on both side, palps, mouth parts of *C. megacephala*



Fig. 66: Lateral view of *Hy. capensis*, shining blackish green color of fly



Fig. 67: Lateral view of *He. illucens*, transparent abdominal segment



Fig. 68: Lateral view of *He. illucens*



Fig. 69: Lateral view of *S. ruficornis*



Fig. 70: Male genitalia of *S. ruficornis*

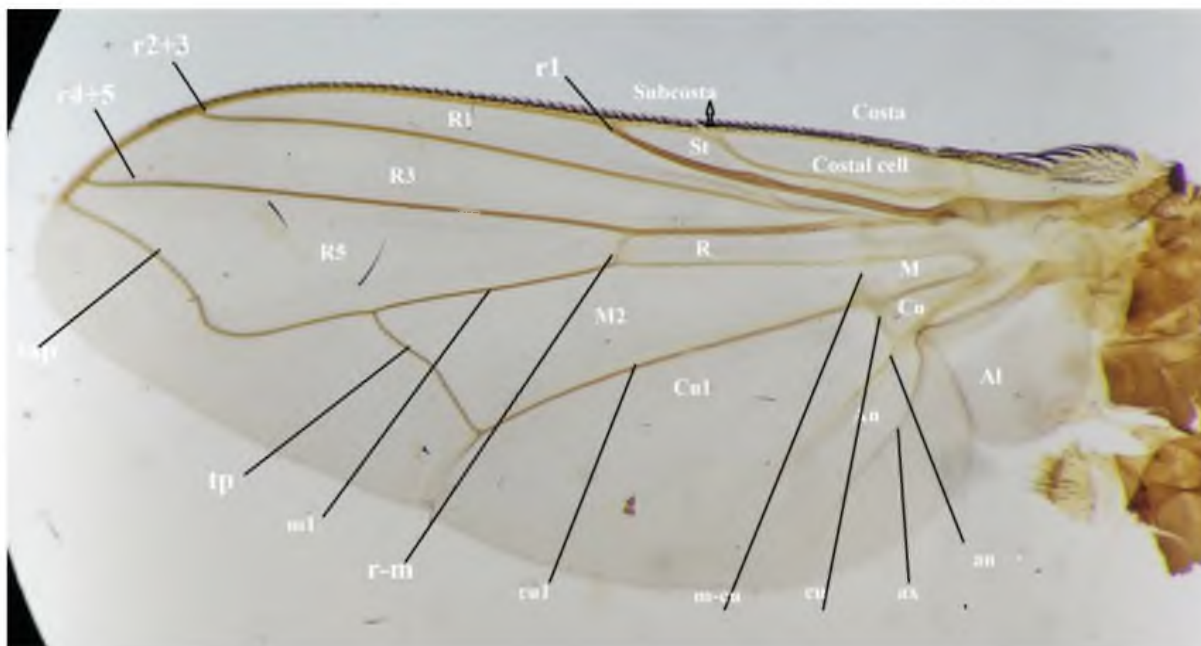


Fig. 71: Wing of *M. domestica*, st: subcostal cell, R: first basal cell, R1: marginal cell, R3: submarginal cell, R5: first posterior cell, M: second basal cell, M2: second posterior or discal cell, Cu: third basal cell, Cu1: third posterior cell, An: axillary cell, Al: alula, r1: first longitudinal vein, r2+3: second longitudinal vein, r4+5: third longitudinal vein, m1: fourth longitudinal vein, cu1: fifth longitudinal vein, an: sixth longitudinal vein, ax: axillary vein: r-m: discal cross vein, m-cu: upper basal cross vein, cu: lower basal cross vein, tap: upper marginal cross vein, tp: lower marginal cross vein



Fig. 72: Wing of *C. megacephala*



Fig. 73: Wing of *Hy. aenescens*



Fig. 74: Wing of *Hy. capensis*

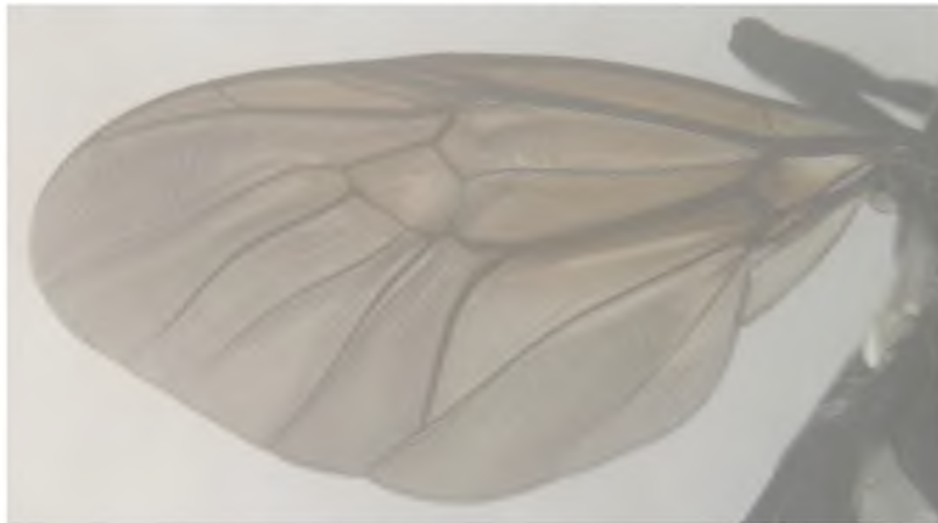


Fig. 75: Wing of *He. illucens*

4.3 Barcoding of flies

The molecular identification (DNA barcoding) was carried out to work out integrated approach for identification of different flies.

PCR amplification of COI (mitochondrial gene) for barcoding

Morphologically six different flies were identified, viz. *M. domestica*, *C. megacephala*, *Hy. aenescens*, *Hy. capensis*, *He. illucens*,. All flies were subjected for barcoding. For barcoding, cytochrome oxidase subunit I mitochondrial gene was targeted and amplified by using universal primers LCO1490/HCO2198 which yielded specific amplicon of 658bp (Fig. 76).

Sequencing of PCR products

The PCR products were sent to M/S Euro fins Ltd. India for sequencing, where PCR products were sequenced in both forward as well as reverse. Sequences were then checked for homology by using the online bioinformatics tool BLAST (Basic local alignment search tool) from NCBI (National centre for Biotechnology information) server which confirmed the specificity of primers. All the sequences for particular species under BLAST system were confirmatory.

Analysis of partial nucleotide sequence of COI gene

The nucleotide sequence data obtained were edited and then subjected for BLAST with NCBI nucleotide sequence data library.

Details of sequences of COI gene for barcoding purpose: A few sequences were edited and submitted to GenBank and accession numbers were allotted and for those species barcode was generated using BoldSystems v3 (Table 7).

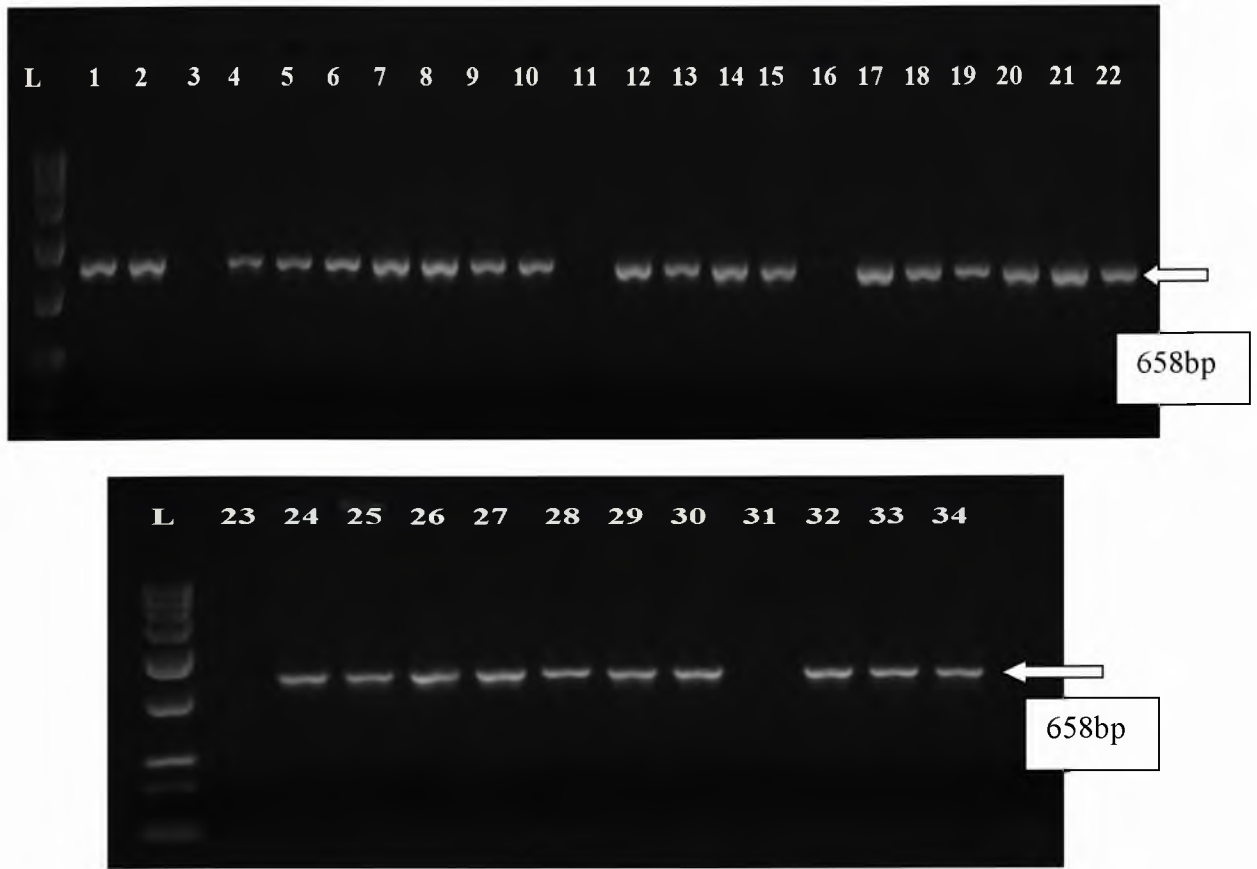
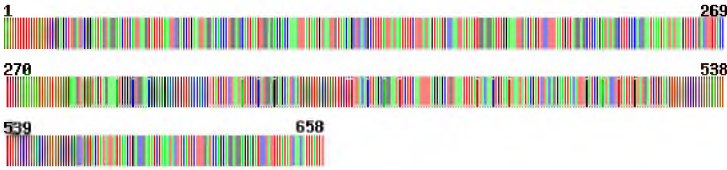
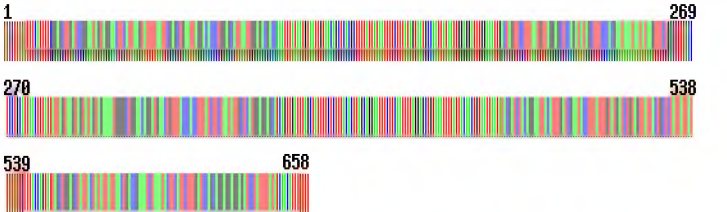
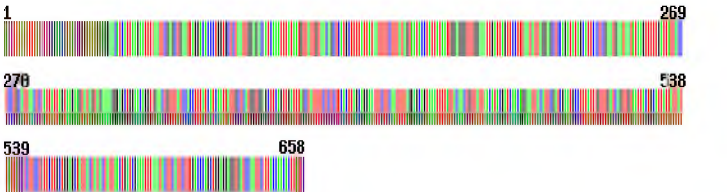
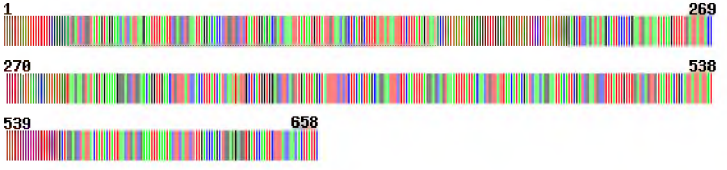
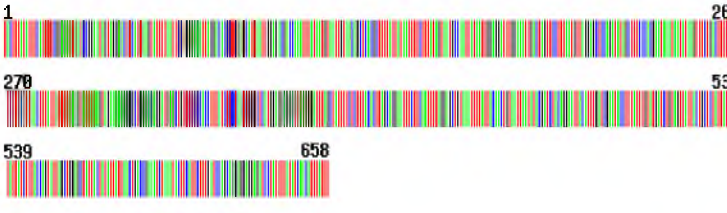
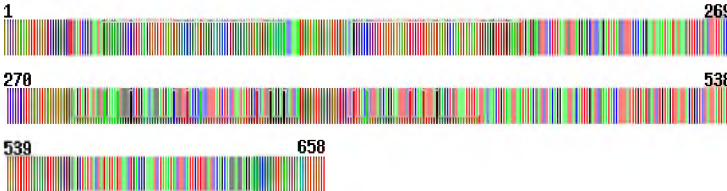


Fig. 76: PCR amplification of COI for barcoding of flies (L: 100 bp Ladder, Lane 1: *C. megacephala*, Lane 2: *C. megacephala*, Lane 3: NTC-no template control, Lane 4: *M. domestica*, Lane 5: *M. domestica*, Lane 6: *C. megacephala*, Lane 7: *C. megacephala*, Lane 8: *M. domestica*, Lane 9: *M. domestica*, Lane 10: *C. megacephala*, Lane 11: NTC, Lane 12: *C. megacephala*, Lane 13: *M. domestica*, Lane 14: : *M. domestica*, Lane 15: : *M. domestica*, Lane 16: NTC, Lane 17: *Hy. capensis*, Lane 18: *C. megacephala*, Lane 19: *M. domestica*, Lane 20: *M. domestica*, Lane 21: *M. domestica*, Lane 22: *M. domestica*, Lane 23: NTC, Lane 24: *M. domestica*, Lane 25: *M. domestica*, Lane 26: *M. domestica*, Lane 27: *M. domestica*, Lane 28: *M. domestica*, Lane 29: *M. domestica*, Lane 30: *M. domestica*, Lane 31: NTC, Lane 32: *He. illucens*, Lane 33: *M. domestica*, Lane 34: *S. ruficornis*)

Table 7: Accession number and barcode ID of different flies

Species	GenBank Accession number	Barcode ID
<i>C. megacephala</i>	>A-2_ KX230656	
<i>C. megacephala</i>	>A-3_ KX230657	
<i>M. domestica</i>	>A-4_ KX230658	
<i>M. domestica</i>	>A-5_ KX230659	
<i>C. megacephala</i>	>A-7_ KX230660	
<i>C. megacephala</i>	>A-8_ KX230661	

<i>M. domestica</i>	>A-10_ KX230662	
<i>M. domestica</i>	>A-11_ KX230663	
<i>C. megacephala</i>	>A-14_ KX230665	
<i>C. megacephala</i>	>A-15_ KX230666	
<i>M. domestica</i>	>A-16_ KX230667	
<i>M. domestica</i>	>A-17_ KX230668	
<i>M. domestica</i>	>A-18_ KX230669	
<i>Hy. capensis</i>	>A-19_ KX230670	

<i>C. megacephala</i>	>A-21_ KX230671	
<i>M. domestica</i>	>A-22_ KX230672	
<i>M. domestica</i>	>A-23_ KX230673	
<i>Musca domestica</i>	>A-24_ KX230674	
<i>M. domestica</i>	>A-26_ KX230676	
<i>M. domestica</i>	>A-27_ KX230677	
<i>M. domestica</i>	>A-29_ KX230678	
<i>M. domestica</i>	>A-30_ KX230679	

<i>M. domestica</i>	>A-31_ KX230680	
<i>Musca domestica</i>	>A-32_ KX230681	
<i>M. domestica</i>	>A-34_ KX230682	
<i>M. domestica</i>	>A-35_ KX230683	
<i>He. illuscens</i>	>A-37_ KX230684	
<i>M. domestica</i>	>A-38_ KX230685	
<i>S. ruficornis</i>	>A-40_ KX230686	

4.4 Evaluation of bio control agents such as entomopathogenic nematodes for fly control

Nematode survival

The survivability of *Steinernema feltiae*, *Heterorhabditis indica*, *S. carpocapsae*, *S. glaseri* and *S. abbasi* was studied in poultry manure for 24 h, 48h, 72h and 96h. *S. feltiae* showed highest survival of about 85.25, 62.75, 48.75 and 23.75% at 24h, 48h, 72h and 96h, respectively, whereas *S. abbasi* showed lowest survivability of 15.25% in 24h and 0% mortality after 48hrs. *Steinernema feltiae* showed good survivability in poultry manure followed by *H. indica*, *S. carpocapsae*, *S. glaseri* and *S. abbasi* (Table. 8; Fig. 77). Analysis of variance showed that there was a significant difference in survival of EPNs in poultry manure ($P < 0.05$) influenced by exposure times ($F = 763.55$; $df = 3, 60$; $P < .0001$), EPN species ($F = 796.17$; $df = 4, 60$; $P < .0001$). The interaction between exposure time and EPN species ($F = 17.31$; $df = 12, 60$; $P < .0001$) was significantly different. Survival of EPNs was drastically reduced with increase in exposing time. Data shows that poultry manure had negative effect on nematode survival.

Further, after exposing these nematodes to poultry manure their virulence capacity was tested against the wax moth, *Galleria mellonella*. All the nematode species were able to cause 100% mortality in *G. mellonella* even after being exposed to the manure for 24h. Progeny production assay revealed that approximately 2.5 lakh IJs were harvested from the cadaver that had been killed by fresh IJs, whereas on an average of 19,000 IJs were harvested from the cadaver that had been killed by IJs after exposing to the poultry manure. These data indicated that poultry manure drastically reduced the survivability of nematodes and also affect the nematode reproduction.

Table 8: Mean percent survival of five EPN species at different time intervals in poultry manure

Hours after treatment	Survival in manure (%)				
	<i>S. feltiae</i>	<i>H. indica</i>	<i>S. carpocapsae</i>	<i>S. glasseri</i>	<i>S. abbasi</i>
24 h	85.25a ^a	83.25a	73a	45a	15.25a
48 h	62.75b	58b	52.25b	9.75b	0
72 h	48.75c	31.25c	28c	2.5c	0
96 h	23.75d	12.25d	14.25d	0	0
<i>P</i> value					
Exposure time (E)	<i>P</i> < 0.0001				
Nematode (N)	<i>P</i> < 0.0001				
E X N	<i>P</i> < 0.0001				

^aMeans of four replications. Means followed by the same letter in a column are not significantly different at $P < 0.05$, as determined by Tukey's test.

Susceptibility of fly egg and pupa on filter paper

The fly eggs and pupae when exposed to *H. indica*, *S. carpocapsae*, *S. glasseri*, *S. abbasi* and *S. feltiae* at 5,000 and 10,000 IJs/host, 24h after nematode inoculation all eggs hatched into larvae (Fig. 78) and in 5 days after nematode inoculation, pupae emerged into adult flies (Fig. 79). This indicated that the eggs and pupae of house fly were resistant to the above five EPNs.

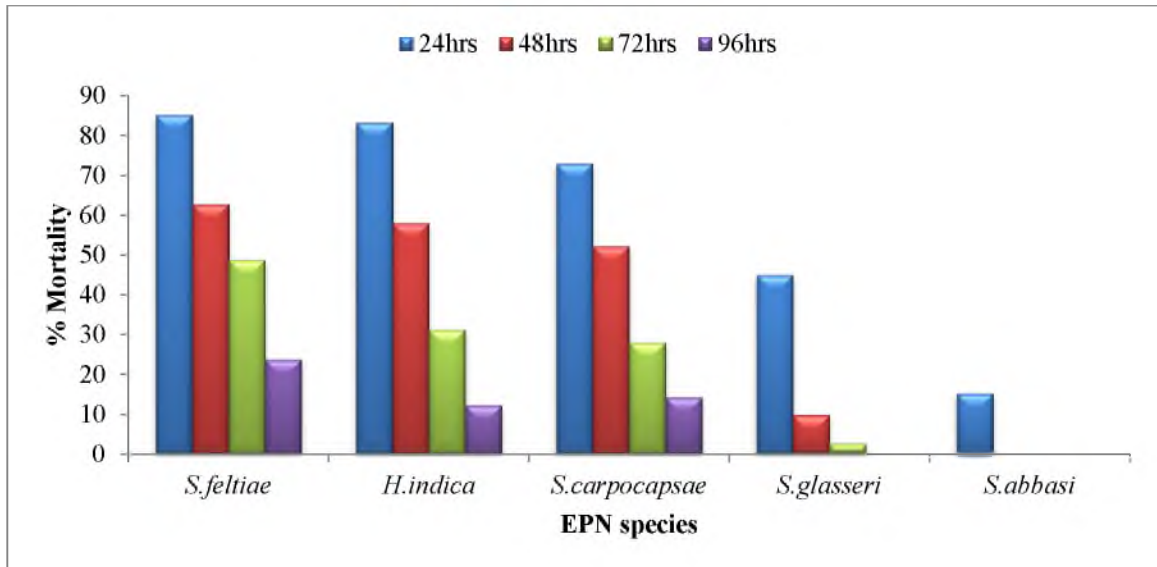


Fig. 77: Mean percent survival of EPNs in poultry manure



Fig. 78: Hatching of nematode inoculated eggs and pupa of housefly

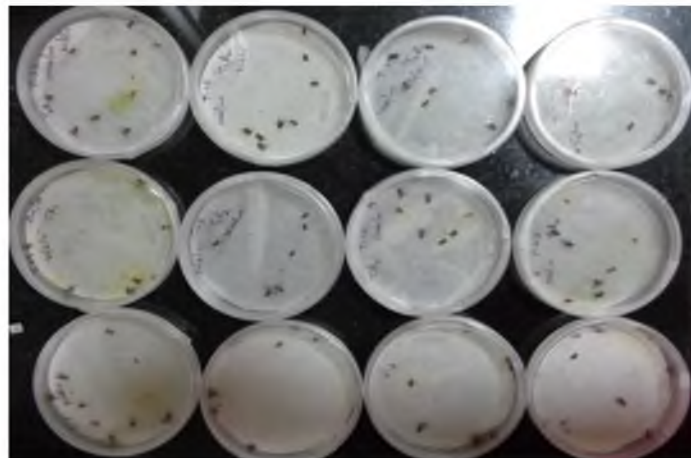


Fig. 79: Hatching of nematode inoculated pupa of housefly

Susceptibility of fly larvae on filter paper

In the present study second and third instar larvae of house fly were susceptible to all the five EPNs viz, *H. indica*, *S. carpocapsae*, *S. glasseri*, *S. abbasi* and *S. feltiae*. *S. carpocapsae* had greater virulence to second instar of house fly at 2 and 3 DAT. *S. carpocapsae* caused highest mortality of 75% (100%), whereas lowest mortality of 0% (12.5%) was caused by *S. abbasi*. at 50 (3000) IJs/larva 2DAT (Fig. 80). At 3DAT, *S. carpocapsae* caused highest mortality of 100%, whereas lowest mortality of 12.5% was observed in *S. abbasi* and *S. feltiae* at 50IJs/larva. Three DAT 100% mortality was recorded in all the EPNs at 3000IJs/larva (Fig. 81).

In this study the analysis of variance showed that the second instar of *M. domestica* mortality was significantly ($P < 0.05$) influenced by IJ concentration ($F = 125.09$; $df = 7, 240$; $P < 0.0001$), EPN species ($F = 201.49$; $df = 4, 240$; $P < 0.0001$), exposure times ($F = 280.99$; $df = 1, 240$; $P < 0.0001$). The interaction between EPN species and IJ concentrations was ($F = 5.69$; $df = 28, 240$; $P = < 0.0001$). The interaction between EPN species, IJ concentrations and exposure time ($F = 4.64$; $df = 35, 240$; $P = < 0.0001$) was significantly different. The calculated lowest LC_{50} and LC_{90} for *S. carpocapsae*, were 19 and 182 at 2DAT whereas LC values could not be calculated for 3DAT because 100% mortality was reached at all doses. The highest LC_{50} and LC_{90} were observed for *S. abbasi* where the LC values could not be calculated for 2DAT because there was no mortality, whereas LC_{50} and LC_{90} values of 309 and 1561 3DAT were observed (Table 9). The LC_{50} and LC_{90} values indicated that *S. carpocapsae* was more virulent against second instars of *M. domestica* compared to other EPNs.

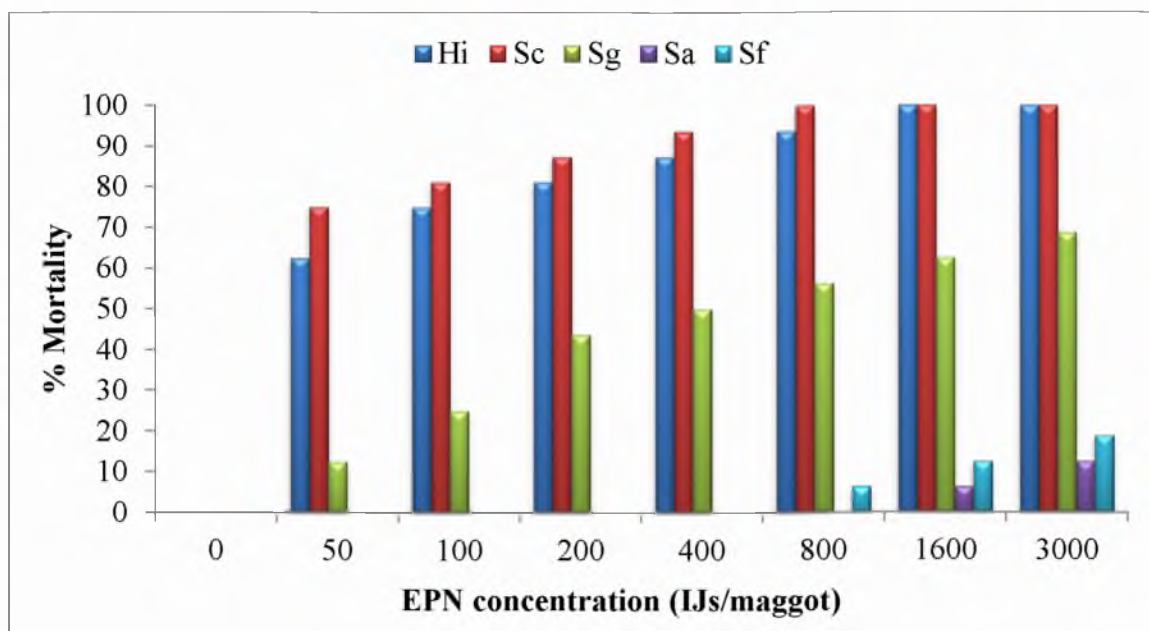


Fig. 80: Mean percent mortality of 2nd instar larvae of *M. domestica* on filter paper to different dose of live EPNs at two days after treatment (DAT), in control there was no mortality (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

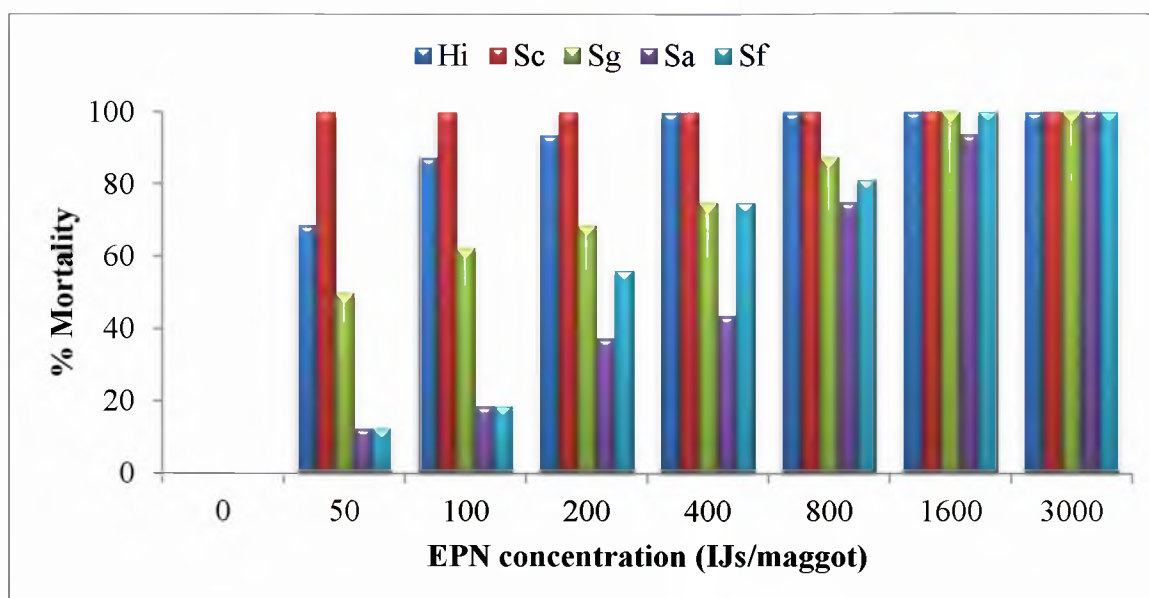


Fig. 81: Mean percent mortality of 2nd instar larvae of *M. domestica* on filter paper to different dose of live EPNs at three days after treatment (DAT), in control there was no mortality (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

Table 9: Dosage mortality studies of 2nd instar larvae of *M. domestica* on filter paper

EPN Species	DAT	LC ₅₀	95% FL	LC ₉₀	95% FL	Slope±SE	χ ²	P
<i>H. indica</i>	2	30	2-68	363	195-1318	1.18±0.33	12.27	0.0005
	3	29	1-54	119	73-383	2.12±0.78	7.33	0.0068
<i>S. carpocapsae</i>	2	19	0-49	182	89-614	1.30±0.44	8.45	0.0037
	3	*	*	*	*	*	*	*
<i>S. glasseri</i>	2	566	280-1387	19261	4782-1083436	0.83±0.21	14.92	0.0001
	3	63	17-117	724	392-2662	1.21±0.28	17.63	<.0001
<i>S. abbasi</i>	2	**						
	3	309	211-447	1561	957-3571	1.82±0.29	39.49	<.0001
<i>S. feltiae</i>	2	8775	3506-605135957	50257	9524-2.61825X10 ¹⁴	1.69±0.74	5.12	0.0237
	3	203	140-283	821	540-1650	2.11±0.34	37.71	<.0001

* Fiducial confidence limits could not be computed for 72h because 100% mortality was observed in all the concentrations. DAT-days after treatment, LC₅₀ and LC₉₀ lethal concentration that kills 50% and 90% of exposed larvae respectively, FL- Fiducial confidence limits, SE- standard error, χ² –chi-square value, P<0.0001highly significant, P<0.05 significant, ** In *S. abbasi* at second day after treatment mortality was not observed

The third instar larvae exposed to all five EPNs lead to *H. indica* causing highest mortality of 81.25% (100%) at 50 (3000) IJs/larva 3DAT whereas least mortality of 0% (0%) was caused by *S. abbasi* at 50 (3000) IJs/larva 3 DAT. *H. indica* causing highest mortality of 81.25% (100%) at 50 (3000) IJs/larva 5DAT whereas least mortality of 0% (62.5%) was caused by *S. abbasi* and *S. glasseri* at 50 (3000) IJs/larva 5 DAT. (Fig. 82 and 83).

Analysis of variance showed that third instar mortality of *M. domestica* was significantly ($P < 0.05$) influenced by IJ concentration ($F = 266.45$; $df = 7, 600$; $P < 0.0001$), EPN species ($F = 524.19$; $df = 4, 600$; $P < 0.0001$), exposure times

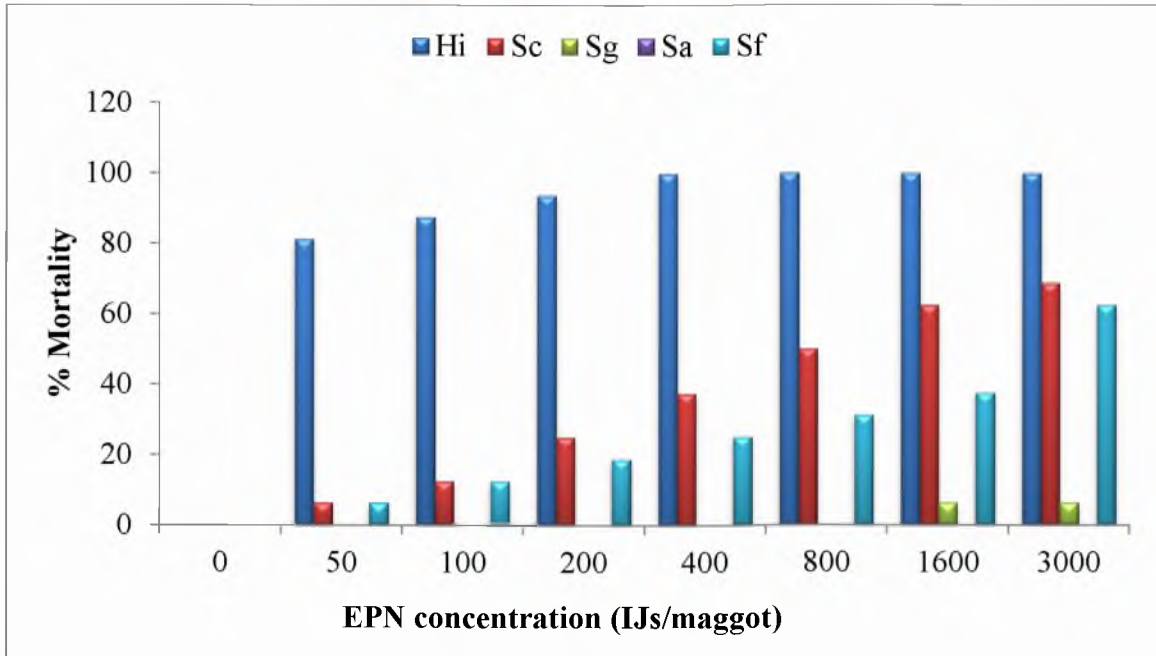


Fig. 82: Mean percent mortality of 3rd instar on filter paper without food three days after treatment with different EPNs at different concentration (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

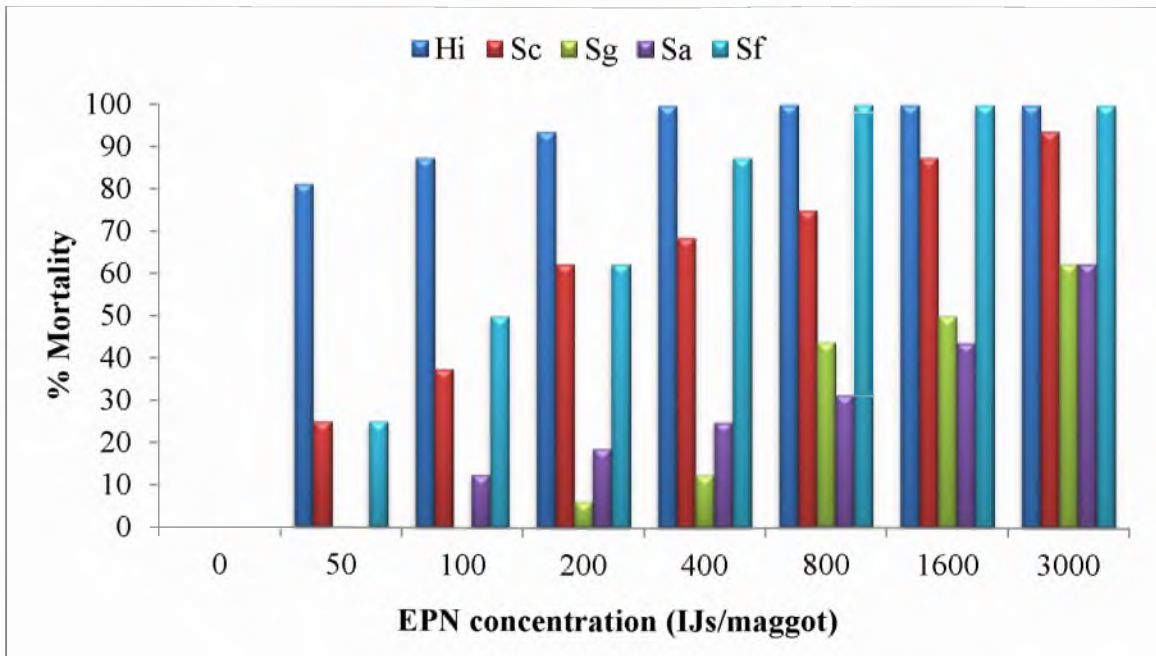


Fig. 83: Mean percent mortality of 3rd instar on filter paper without food five days after treatment with different EPNs at different concentration (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

($F = 424.44$; $df = 4, 600$; $P < 0.0001$). The interaction between EPN species and IJ concentrations ($F = 13.76$; $df = 28, 600$; $P < 0.0001$). The interaction between EPN species, IJ concentrations and exposure time ($F = 5.11$; $df = 140, 600$; $P < 0.0001$) was significantly different. The lowest LC_{50} and LC_{90} against third instar larvae was observed for *H. indica* were 814 (16) and 4363(101) 3 (5) DAT, however the LC values could not be calculated for *S. abbasi* as there was no mortality 3DAT. The highest LC_{50} and LC_{90} were found for *S. abbasi* were 1798 and 26477 5DAT (Table 11). The LC_{50} and LC_{90} values indicated that *H. indica* was more virulent against third instars of *M. domestica*.

Table 10: Dosage mortality studies of 2nd instar larvae of *M. domestica* to EPNs on filter paper with artificial diet

EPN Species	DAT	LC_{50}	95% FL	LC_{90}	95% FL	Slope±SE	χ^2	P
<i>H. indica</i>	3	88	25-165	1480	703-8242	1.04±0.25	17.47	<.0001
	5	41	9-76	295	175-847	1.51±0.40	14.15	0.0002
<i>S. carpocapsae</i>	3	1109	602-3186	23930	6322-799429	0.96±0.23	17.45	<.0001
	5	144	73-231	1321	720-4257	1.33±0.26	25.86	<.0001
<i>S. glasseri</i>	3	996	669-1664	6068	3095-22324	1.63±0.29	30.89	<.0001
	5	171	112-245	804	511-1752	1.91±0.32	34.18	<.0001
<i>S. abbasi</i>	3	2453	1502-6411	15327	6015-160161	1.61±0.37	18.81	<.0001
	5	378	230-612	3655	1790-14642	1.30±0.24	29.28	<.0001
<i>S. feltiae</i>	3	8775	3506-605135957	50257	9524-2.61825X10 ¹⁴	1.69±0.74	5.12	0.0237
	5	3482	1424-51533	116441	15332-187114667	0.84±0.24	11.43	0.0007

DAT-days after treatment, LC_{50} and LC_{90} lethal concentration that kills 50% and 90% of exposed larvae, respectively, FL- Fiducial confidence limits, SE-standard error, χ^2 –chi-square value, $P < 0.0001$ highly significant, $P < 0.05$ significant

Signs of nematode infection:

After causing mortality of house fly larvae by *H. indica* the cadaver turned into brownish colour (Fig. 84) and in case of *S. feltiae* cadaver turned into black colour (Fig. 85). Whereas *S. carpocapsae*, *S. glasseri*, *S. abbasi* did not change colour and remained as creamy white (Fig. 86). After 3 days, the penetrated infective juveniles (IJs) developed into adults in dead cadaver (Fig. 87) and after 7 and 10 days of mortality of larvae, IJs (Fig. 88-90) developed into adults inside the cadaver for *Steinernema* and *Heterorhabditis*, respectively. The confirmation of larvae which were dead because of EPNs or natural death were ascertained by dissecting the dead larvae after 3 days of death to check for adults inside the cadaver (Fig. 91-93).

Susceptibility of fly larvae on filter paper with artificial diet

The results of susceptibility of second instar larvae to EPNs on filter paper with artificial diet was similar to that without providing diet to larvae on filter paper but when larvae were provided with diet there was a decrease in percent mortality. Three DAT, 100% mortality was observed in *H. indica* whereas the lowest mortality of 18.75% was observed in *S. feltiae* at 3000IJs/larva (Fig. 94). Hundred percent mortality was observed in *H. indica*, *S. carpocapsae* and *S. glasseri*. However only 43.75% was observed for *S. feltiae* at 3000IJs/larva 5DAT (Fig. 95).

The Analysis of variance observed for second instar of *M. domestica* mortality was significantly ($P < 0.05$) influenced by IJ concentration ($F = 298.32$; $df = 7, 600$; $P < 0.0001$), EPN species ($F = 293.58$; $df = 4, 600$; $P < 0.0001$), exposure times ($F = 266.18$; $df = 4, 600$; $P < 0.0001$). The interaction between EPN species and IJ concentrations



Fig. 84: Healthy, Fig. 85: *H. indica*, Fig. 86: *S. feltiae*, Fig. 87: *S. carpocapsae* infected larva of *M. domestica*



Fig. 88: *H. indica* Fig. 89: *S. feltiae* Fig. 90: *S. carpocapsae* adults in *M. domestica* larva

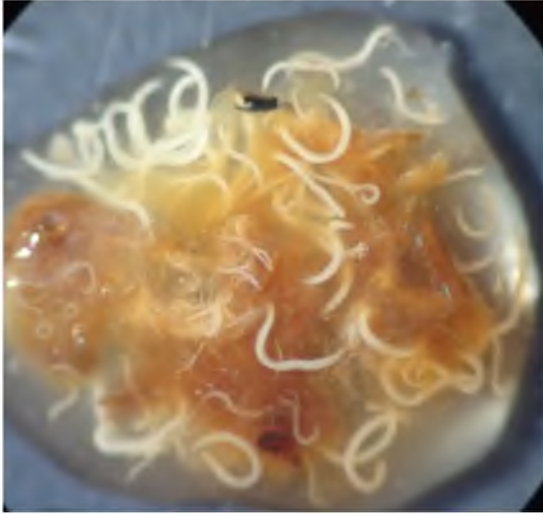


Fig. 91: *H. indica*

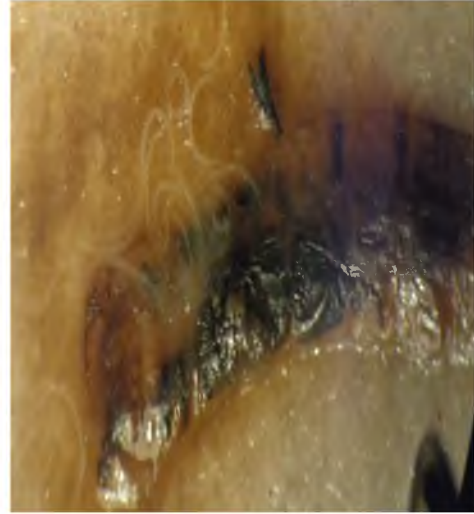


Fig. 92: *S. feltiae*

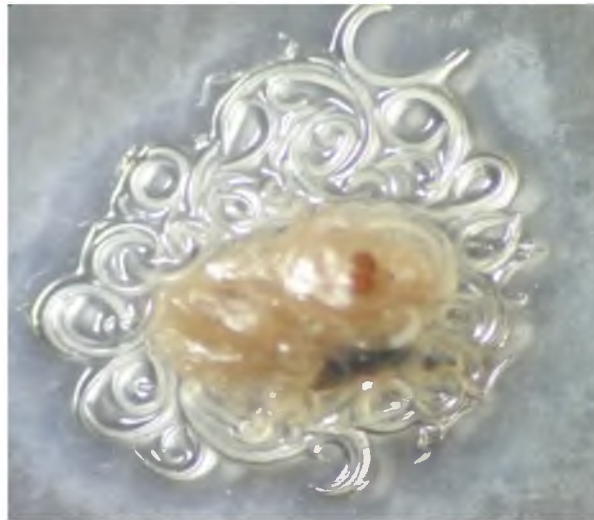


Fig. 93: *S. carpocapsae* –Dissected *M. domestica* larva containing adults of EPNs

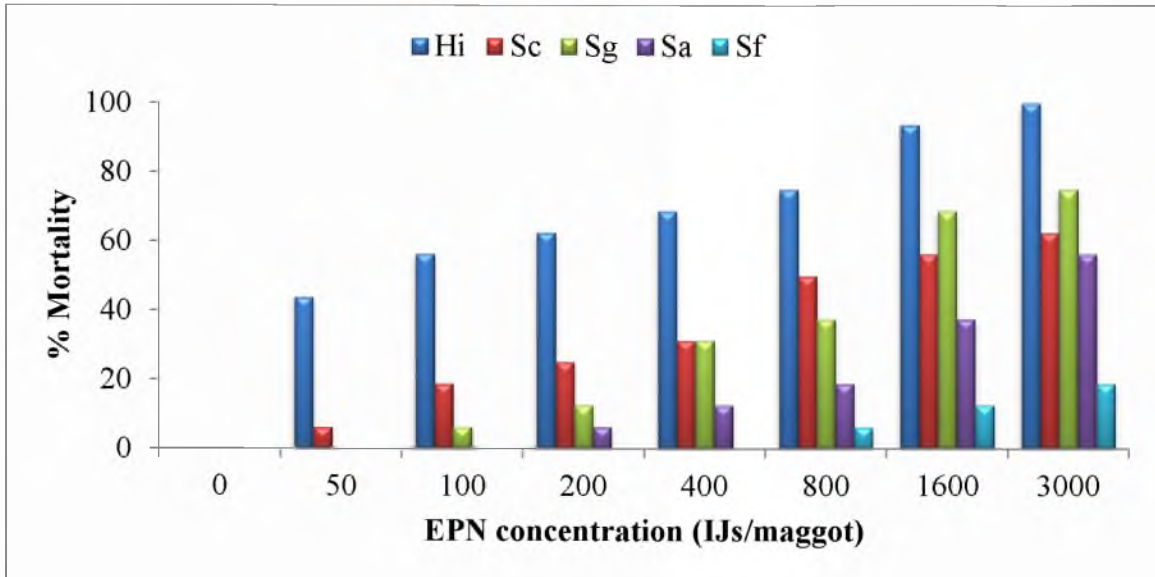


Fig. 94: Mean percent mortality of 2nd instar larvae of *M. domestica* on filter paper provided with artificial food three day after treatment of different EPNs at different concentrations (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

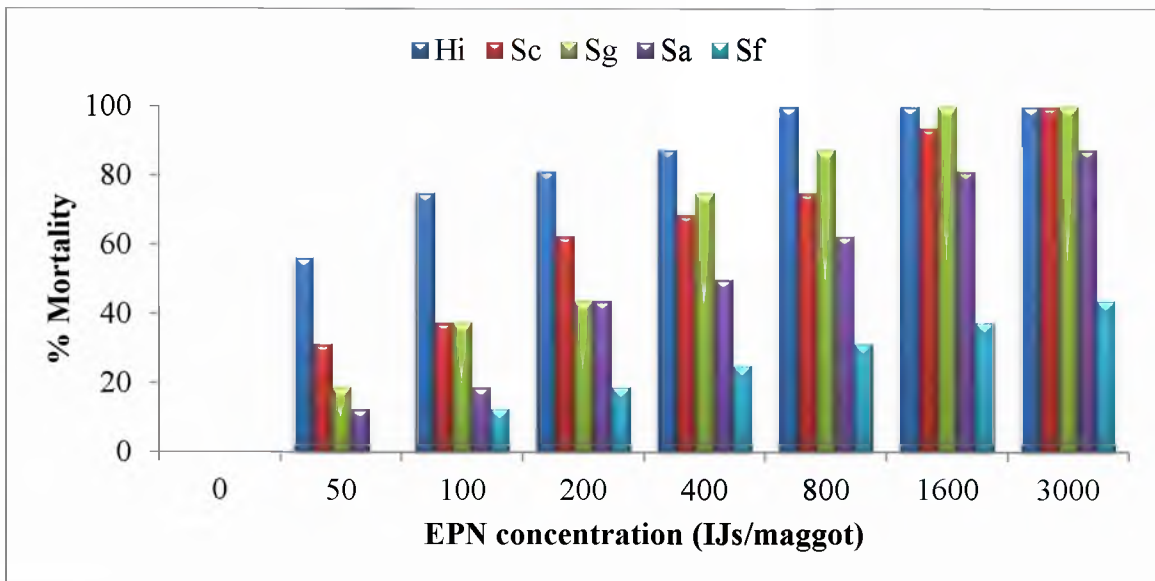


Fig. 95: Mean percent mortality of 2nd instar larvae of *M. domestica* on filter paper provided with artificial food five day after treatment of different EPNs at different concentrations (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

Table 11: Dosage mortality studies of 3rd instar of *M. domestica* with EPNs on filter paper

EPN Species	DAT	LC ₅₀	95% FL	LC ₉₀	95% FL	Slope±SE	χ^2	P
<i>H. indica</i>	3	814	560-1259	4363	2433-12824	1.75±0.30	33.71	<.0001
	5	16	0-42	101	32-377	1.62±0.70	5.32	0.0211
<i>S. carpocapsae</i>	3	886	526-1862	11921	4309-118606	1.13±0.23	22.71	<.0001
	5	163	77-271	1983	987-8224	1.18±0.24	23.81	<.0001
<i>S. glasseri</i>	3	**						
	5	1560	1045-2853	8394	4096-38998	1.75±0.35	24.85	<.0001
<i>S. abbasi</i>	3	**						
	5	1798	982-5837	26477	7403-701938	1.09±0.25	18.30	<.0001
<i>S. feltiae</i>	3	2197	1051-12364	55536	10611-8104407	0.91±0.24	14.46	0.0001
	5	110	70-154	416	277-881	2.21±0.43	26.49	<.0001

** Mortality was not recorded at three day after treatment of EPNs in third instar larvae of *M. domestica*. DAT-days after treatment, LC₅₀ and LC₉₀ lethal concentration that kills 50% and 90% of exposed larvae, respectively, FL- Fiducial confidence limits, SE-standard error, χ^2 —chi-square value, P<0.0001highly significant, P<0.05 significant

($F = 8.57$; $df = 28, 600$; $P < 0.0001$). The interaction between EPN species, IJ concentrations and exposure time ($F = 2.77$; $df = 140, 600$; $P < 0.0001$) was significantly different. The calculated lowest LC_{50} and LC_{90} were for *H. indica*, on second instar house fly larvae with artificial diet 3 (5) DAT were 88 (41) and 1480 (295), whereas lowest of 8775 (3482) and 50257(116441) for *S. feltiae* (Table. 10). The LC_{50} and LC_{90} values indicated that *H. indica*, was more virulent against second instars of *M. domestica* with artificial diet.

Mortality of third instar larvae when exposed to all the EPN in artificial diet, *H. indica* showed highest mortality of 56.25 (100%), whereas *S. feltiae* showed lowest mortality of 12.25(37.5%) at 3000IJs/larva 3(5) DAT (Fig. 96 and 97).

Analysis of variance observed that third instar of *M. domestica* with artificial diet mortality was significantly ($P < 0.05$) influenced by IJ concentration ($F = 92.49$; $df = 7, 480$; $P < 0.0001$), EPN species ($F = 111.58$; $df = 4, 480$; $P < 0.0001$), exposure times ($F = 57.75$; $df = 3, 480$; $P < 0.0001$). The interaction between EPN species and IJ concentrations ($F = 3.03$; $df = 28$; $P < 0.0001$). The interaction between EPN species, IJ concentrations and exposure time ($F = 0.63$; $df = 105, 480$; $P = 0.9977$) was not significantly different. The calculated lowest LC_{50} and LC_{90} for *H. indica*, on third instar housefly larvae 3 (5) DAT were 1456 (69) and 51270 (1525), whereas the LC values could not be calculated for *S. feltiae* as there was no mortality 3DAT, the lowest LC_{50} and LC_{90} were for *S. feltiae* 5241 and 135607 5DAT (Table. 12). The LC_{50} and LC_{90} values indicated that *H. indica* was more virulent against third instars of *M. domestica*.

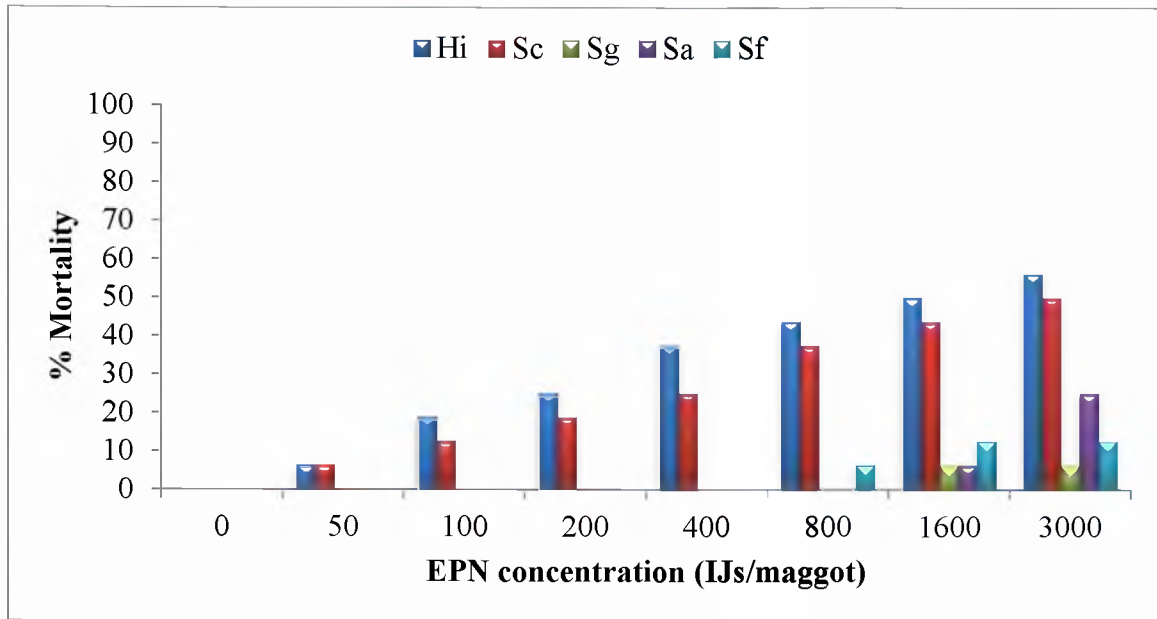


Fig. 96: Mean percent mortality of 3rd instar larvae of *M. domestica* on filter paper with artificial food at three days after treatment with different dose of different EPNs (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

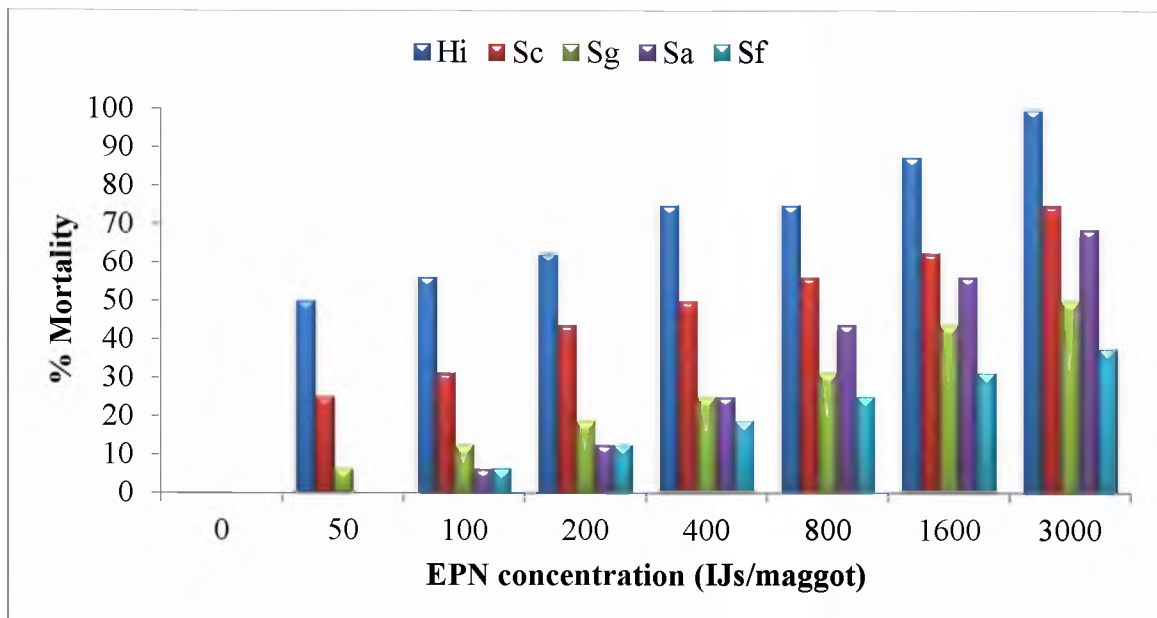


Fig. 97: Mean percent mortality of 3rd instar larvae of *M. domestica* on filter paper with artificial food at five days after treatment with different dose of different EPNs (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

Table 12: Dosage mortality of 3rd instar larvae of *M. domestica* with EPNs on filter paper with artificial food

EPN Species	DAT	LC ₅₀	95% FL	LC ₉₀	95% FL	Slope±SE	χ^2	P
<i>H. indica</i>	3	1456	708-7033	51270	9278-11606858	0.82±0.22	13.41	0.0002
	5	69	13-141	1525	685-11362	0.95±0.24	14.94	0.0001
<i>S. carpocapsae</i>	3	2487	1103-21299	84453	12823-47568034	0.83±0.23	12.54	0.0004
	5	429	171-1154	27175	5122-10387651	0.71±0.21	11.41	0.0007
<i>S. glasseri</i>	3	**						
	5	2772	1185-29611	100336	13994-95961730	0.82±0.23	12.01	0.0005
<i>S. abbasi</i>	3	4568	3002-9.7	10437	4808-2.4644X10 ³⁶	3.57±1.7	4.00	0.0456
	5	1216	785-2286	8918	4011-46833	1.48±0.28	26.85	<.0001
<i>S. feltiae</i>	3							
	5	5241	1989-128117	135607	1716-336567111	0.90±0.27	10.92	0.0010

** The mortality was not recorded in third instar larvae of *M. domestica* against *S. glasseri* at three days after treatment. DAT-days after treatment, LC₅₀ and LC₉₀ lethal concentration that kills 50% and 90% of exposed larvae respectively, FL- Fiducial confidence limits, SE-standard error, χ^2 –chi-square value, P<0.0001highly significant, P<0.05 significant

All EPN species caused significantly ($P < 0.05$) higher mortality with increasing IJs concentrations. Five DAT irrespective of the IJs concentrations, the mortality of both house fly larval stages were significantly ($P < 0.05$) increased compared with the 3 DAT. Among the larval stages, irrespective of EPN species, second larval stage was significantly ($P < 0.05$) more susceptible compared with third instar larva of housefly.

Susceptibility of fly larvae in poultry manure

When comparing the efficacy of EPNs against larvae of *M. domestica* in plastic containers containing poultry manure, larval mortality could not be noticed at even 50-3000IJs/larva. Therefore EPNs dose was increased to 6000; 7000; 8000; 9000; 10000; 12000; 14000; 16000; 18000; 20000; 32000; 64000; 128000 and 256000IJs/larva. *H. indica* caused 6.25% mortality of second instar at 16000 IJs/larva, whereas *H. indica* caused 6.25% mortality of third instar at 32000 IJs/larva. *S. carpocapsae* caused 6.25% mortality of second instar at 64000 IJs/larva, whereas *S. carpocapsae* caused 6.25% mortality third instar at 128000/larva. Other three EPNs *S. glasseri*, *S. abbasi* and *S. feltiae* did not cause any mortality in both second and third instar larvae even at the highest dose of 256000IJs/larva (Fig. 98 and 99).

Progeny production

The progeny production assay revealed that all the five nematodes were able to reproduce in the insect larvae and produce IJs 1240, 1100, 595, 570 and 1080IJs larva⁻¹ respectively. *H. indica* produced significantly more number of infective juveniles per insect larva than the other nematode species. Among the five EPNs studied, the least progeny production was recorded for *S. abbasi* (Fig. 100-103).

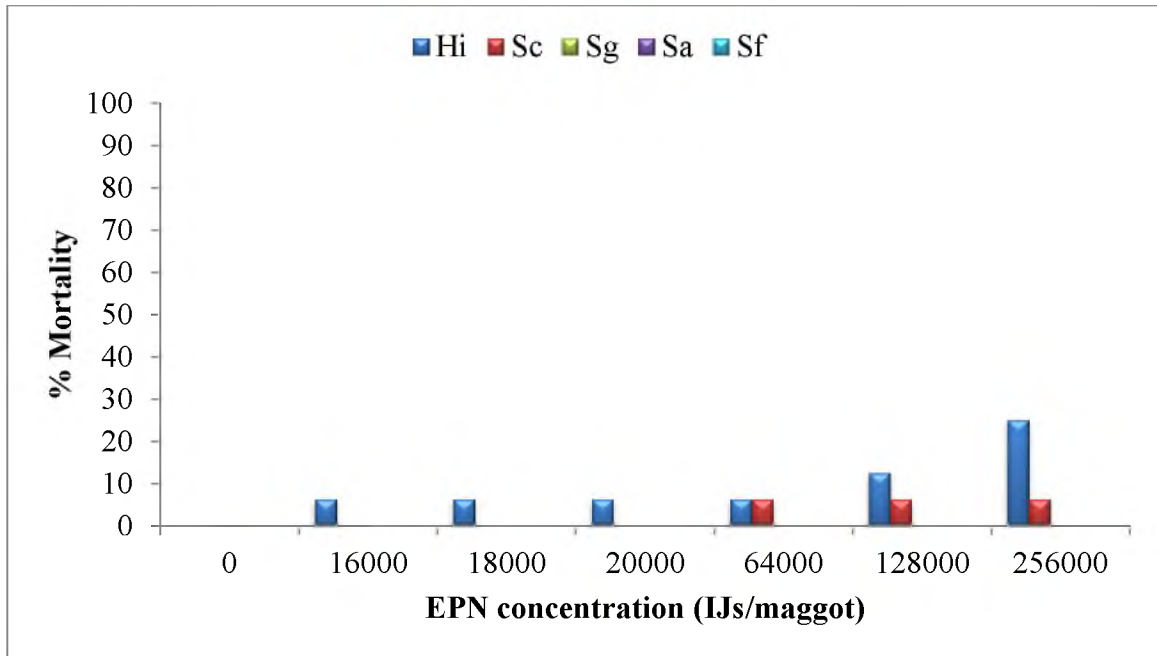


Fig. 98: Mean percent mortality of 2nd instar larvae of *M. domestica* against different EPN dose in poultry manure at two days after treatment (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)

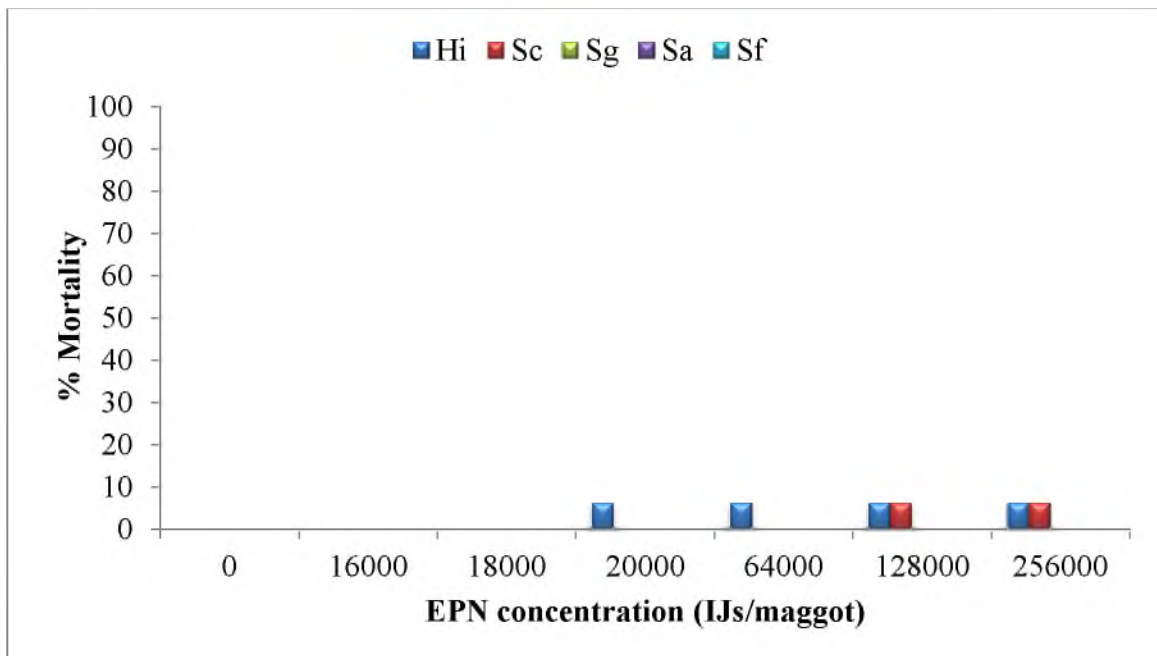


Fig. 99: Mean percent mortality of 3rd instar larvae of *M. domestica* against different EPN dose in poultry manure at two days after treatment (Hi=*H. indica*, Sc=*S. carpocapsae*, Sg=*S. glasseri*, Sa=*S. abbasi*, Sf=*S. feltiae*)



Fig. 100: *H. indica* –reproduction of EPN IJs in *M. domestica* larva



Fig. 101: *S. carpocapsae* -reproduction of EPN IJs in *M. domestica* larva

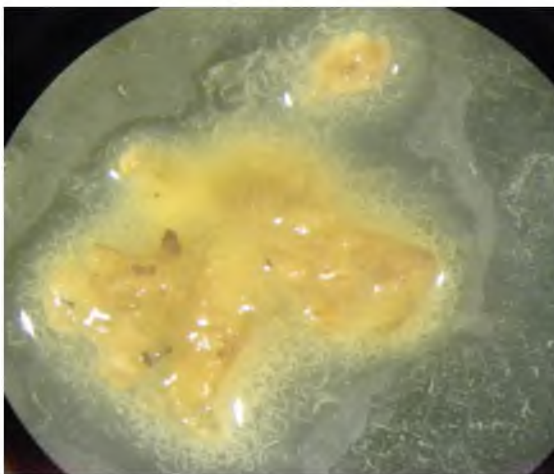


Fig. 102: *H. indica*



Fig. 103: *S. carpocapsae* -progeny production in *M. domestica* larva

4.5 Evaluation of biopesticides against fly control

This study had been undertaken to find the efficiency of sweet basil (*Ocimum basilicum*), cumin seed (*Cuminum cyminum*), neem (*Azadirachta indica*) and curcumin (*Curcuma longa*) at different concentration on different developmental stages of houseflies. Lethal doses for fumigation, topical applications, feed toxicity and residual toxicity were detected. Taking the lethal doses found from these tests is an important parameter of the biological potency for the selection of the essential oils which are suitable for the development of possible insecticides. The present study focused on effectiveness based on mortality after the application within 24 h of the application.

Fumigation-adulticidal assay

Adulticidal activity of basil, cumin seed essential oils through fumigant toxicity assay was found to be dose and time dependent where the mortality increased with increase in the concentration and time (Fig. 104 and 105). Lethal concentration, LC₅₀ and LC₉₉ at the exposure period of 2h was 0.030mg/cm³ and 0.074 mg/cm³, respectively for basil and 0.018mg/cm³ and 0.057 mg/cm³, respectively for cumin seed. The LC values of cumin seed were lower than the basil suggesting cumin seed is more effective than basil against adults. There was no fumigant toxicity of neem and curcumin on adults leading to absence of mortality.

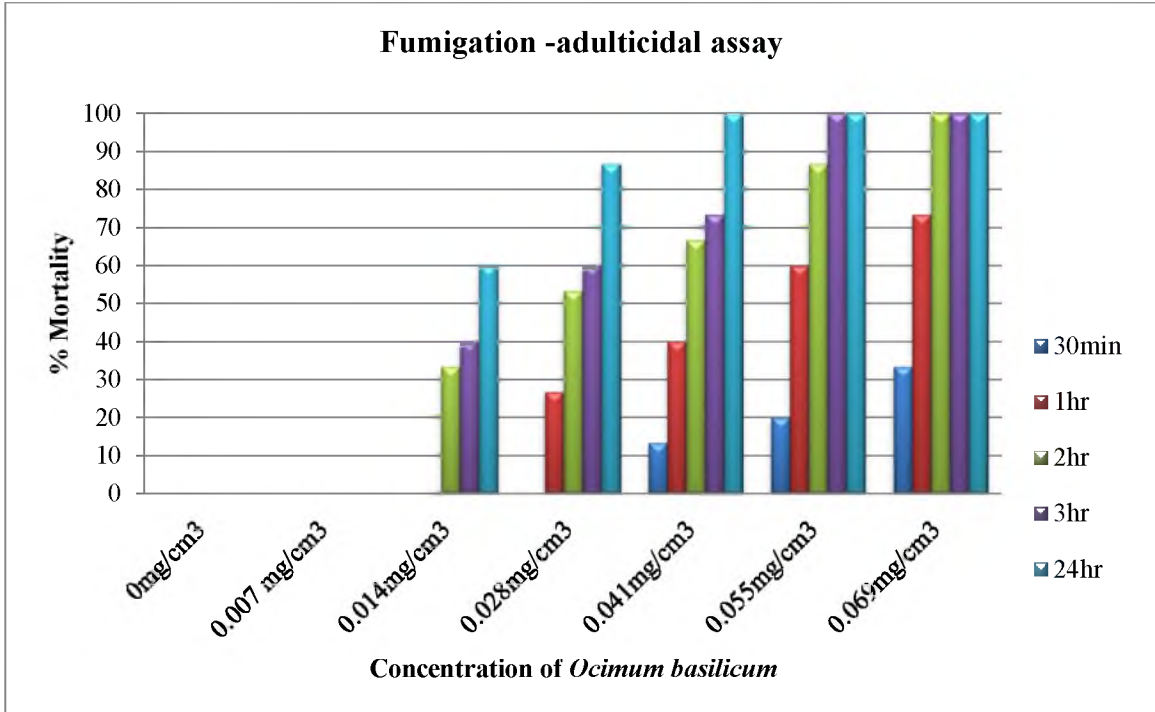


Fig. 104: Fumigant toxicity of *M. domestica* adults with *O. basilicum*

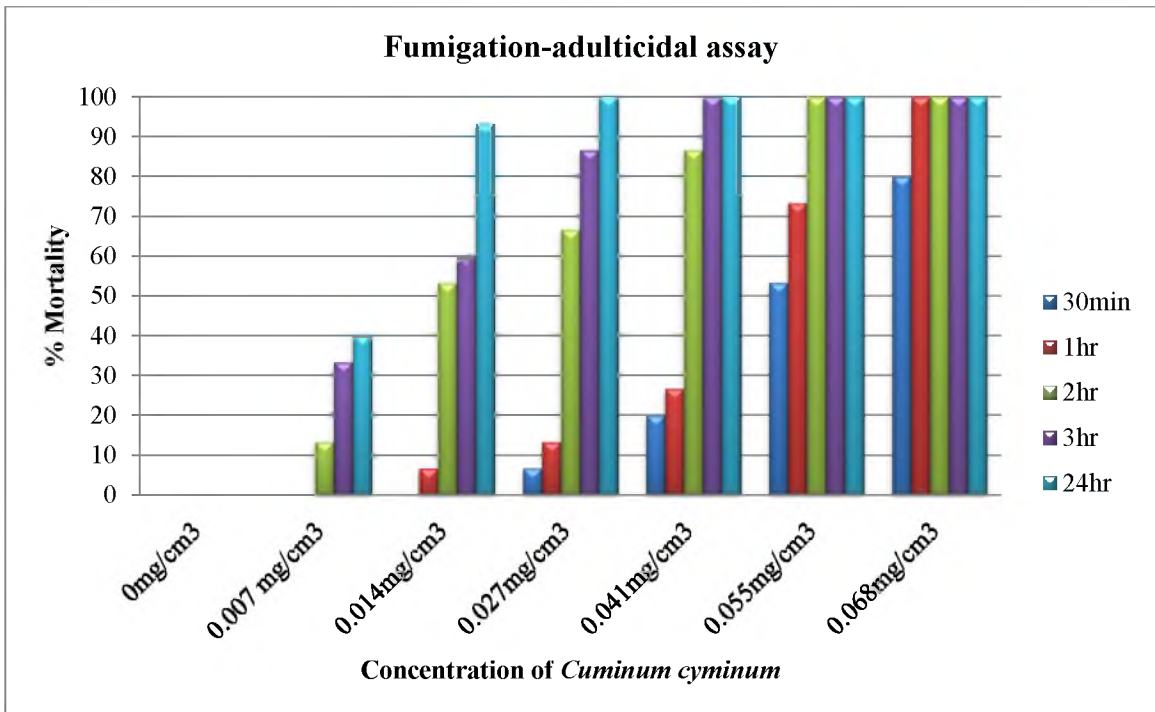


Fig. 105: Fumigant toxicity of *M. domestica* adults with *C. cyminum*

Fumigation-larvicidal assay

Larvicidal activity of basil, cumin seed essential oils through fumigant toxicity assay was found to be dose and time dependent where the mortality increased in proportion to the increase in the concentration and time (Fig. 106 and 107). Lethal concentration, LC_{50} and LC_{99} at the exposure period 24h was $0.10\text{mg}/\text{cm}^3$ and $0.24\text{mg}/\text{cm}^3$, respectively for basil and $0.08\text{mg}/\text{cm}^3$ and $0.14\text{mg}/\text{cm}^3$, respectively for cumin seed. The LC values of cumin seed were lower than the basil suggesting cumin seed was more effective than basil against larvae. There was no fumigant toxicity of neem and curcumin on larvae and no mortality was observed.

Fumigation-pupicidal assay

Pupicidal assay with basil, cumin seed essential oil against housefly pupae resulted in a PIR value between 0% to 100% for basil and 0% to 66.67% for cumin seed at different concentrations of oil tested (Table. 13). Lethal concentration, LC_{50} and LC_{99} was $0.07\text{mg}/\text{cm}^3$ and $0.14\text{mg}/\text{cm}^3$, respectively for basil and $0.11\text{mg}/\text{cm}^3$ and $0.20\text{mg}/\text{cm}^3$, respectively for cumin seed. The LC values of basil were lower than cumin seed indicating that basil was more effective than cumin seed against pupae. There was no pupicidal activity of two essential oils of neem and curcumin since all the pupae emerged into adults in all treated and control trials.

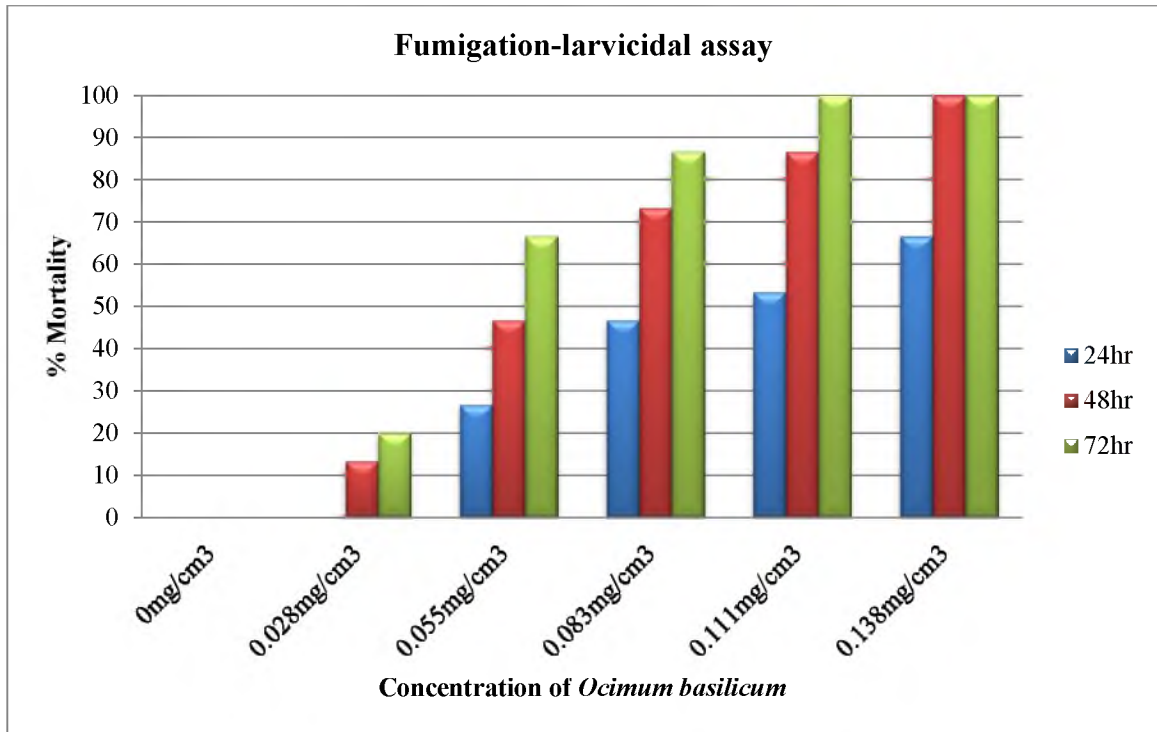


Fig. 106: Fumigant toxicity of *M. domestica* larva with *O. basilicum*

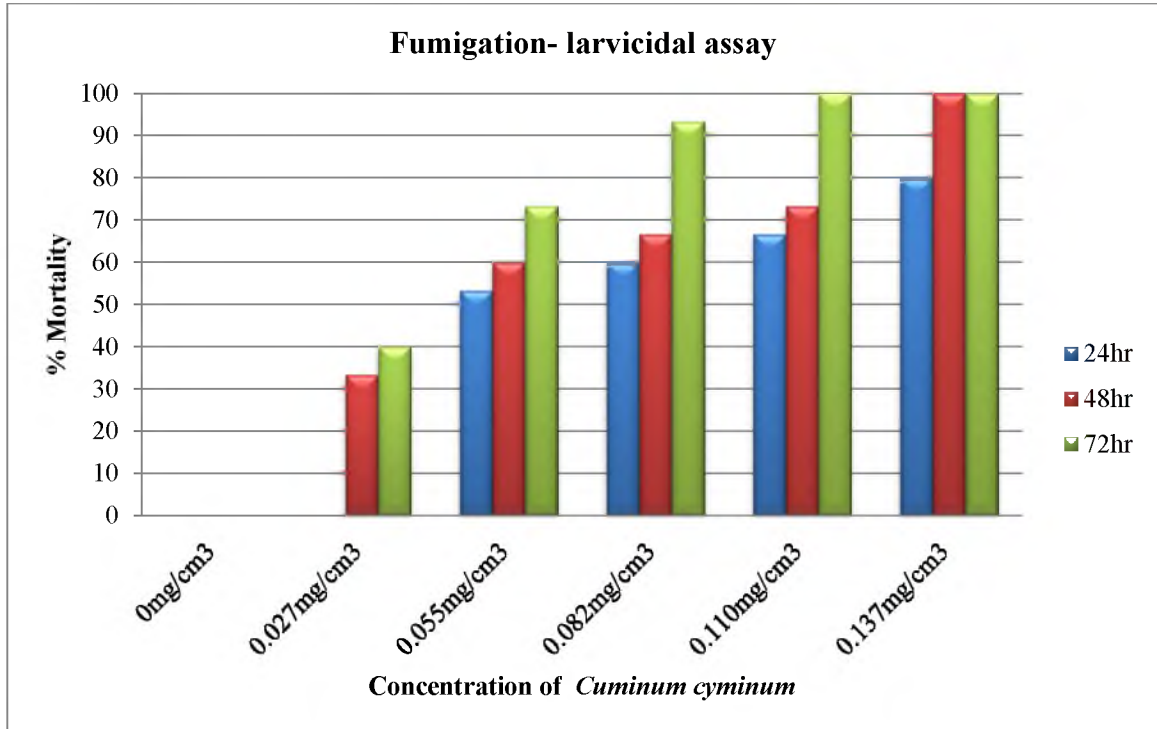


Fig. 107: Fumigant toxicity of *M. domestica* larva with *C. cyminum*

Table 13: Percent inhibition of adult emergence from pupa with different concentration of basil and cumin seed

Treatment (mg/cm ³)	Basil	Treatment (mg/cm ³)	Cumin seed
0	0	0	0
0.028	0	0.027	0
0.055	53.33	0.055	0
0.083	60	0.082	26.67
0.111	86.67	0.110	53.3
0.138	100	0.137	66.67

Fumigation-ovicidal assay

Fumigation-ovicidal assay with basil, cumin seed essential oil against housefly pupae resulted in a PIR value from 0% to 100% for basil and 0% to 73.33% for cumin seed at different concentrations of oil tested (Table 14). Basil was found more effective than cumin seed against eggs (Fig. 108). There was no ovicidal activity of neem and curcumin in these two essential oils since all the eggs emerged into larvae in all treated and control groups.

Table 14: Percent inhibition of larval emergence from eggs with different concentration of basil and cumin seed

Treatment (μl)	Basil	Treatment (mg/cm ³)	Cumin seed
0	0	0	0
0.028	0	0.027	0
0.055	46.67	0.055	0
0.083	53.33	0.082	26.67
0.111	86.67	0.110	46.67
0.138	100	0.137	73.33

Contact toxicity (topical) adulticidal assay

Adulticidal activity of basil, cumin seed essential oil through topical contact toxicity assay was found to be dose and time dependent. The percent mortality of adults with topical assay is given in (Fig. 109 and 110). Lethal concentration, LC_{50} and LC_{99} at the exposure period of 2h was $0.046\text{mg}/\text{cm}^3$ and $0.06\text{ mg}/\text{cm}^3$, respectively for basil and $0.059\text{mg}/\text{cm}^3$ and $0.09\text{ mg}/\text{cm}^3$, respectively for cumin seed. The LC values of basil were lower than the cumin seed suggesting that basil was more effective than cumin seed against adults. There was no topical contact toxicity of neem and curcumin on adults leading to no mortality of flies.

Larvicidal activity of basil, cumin seed essential oil through topical contact toxicity assay was found to be dose and time dependent. The percent mortality of larvae with topical assay is given in the (Fig. 111 and 112). Lethal concentration, LC_{50} and LC_{99} at the exposure period 2h was $0.184\text{mg}/\text{cm}^3$ and $0.50\text{ mg}/\text{cm}^3$, respectively for basil and $0.137\text{mg}/\text{cm}^3$ and $0.39\text{ mg}/\text{cm}^3$, respectively for cumin seed. The LC values of cumin seed were lower than the basil suggesting cumin seed was more effective than basil against larvae. There was no topical contact toxicity of neem and curcumin on adults leading to no mortality.

Contact toxicity pupicidal assay

Pupicidal assay with basil, cumin seed essential oil against housefly pupae resulted in a PIR value from 0% to 100% for basil and 0% to 100% for cumin seed at different concentrations of oil tested (Table 15).

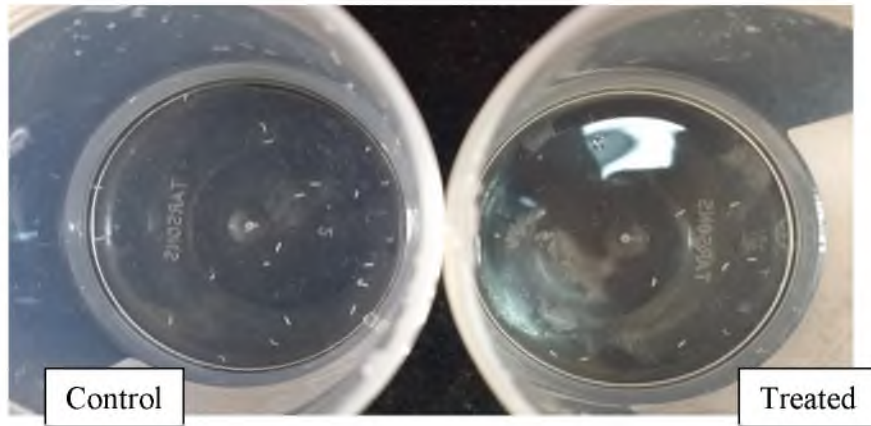


Fig. 108: Ovicidal assay of eggs

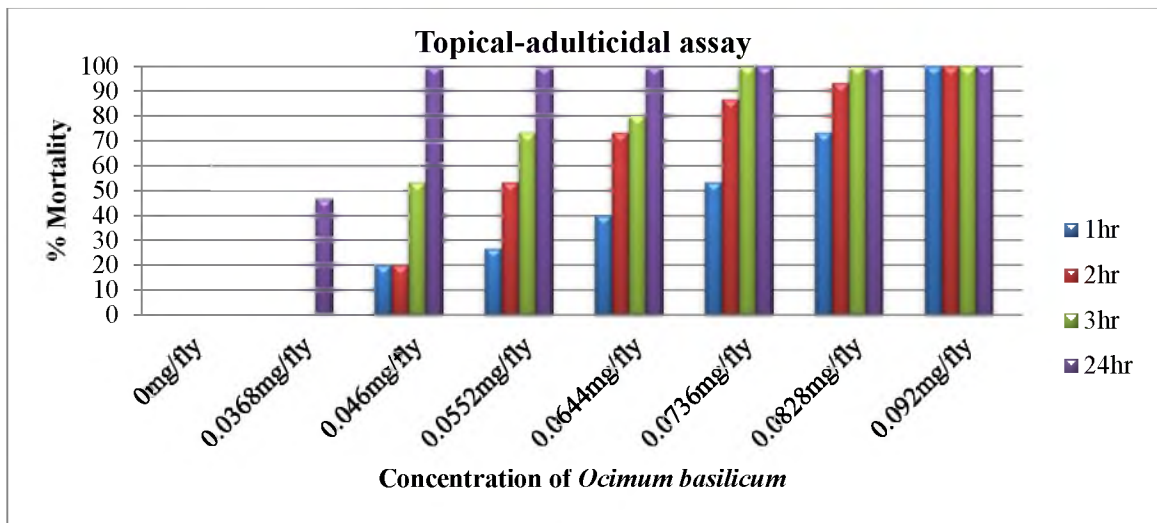


Fig. 109: Contact toxicity of *M. domestica* adults with *O. basilicum*

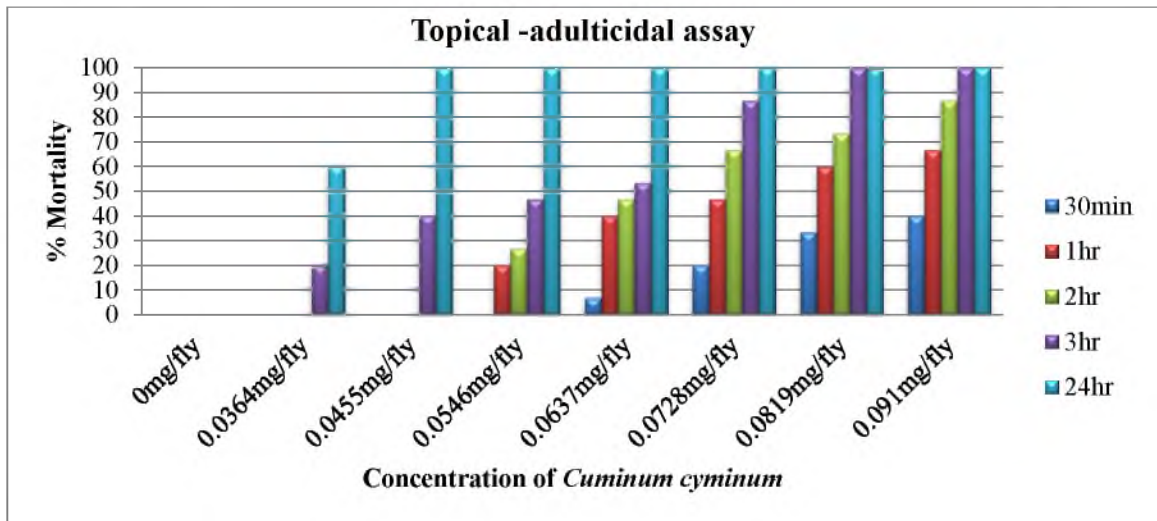


Fig. 110: Contact toxicity of *M. domestica* adults with *C. cyminum* Contact toxicity (topical) larvicidal assay

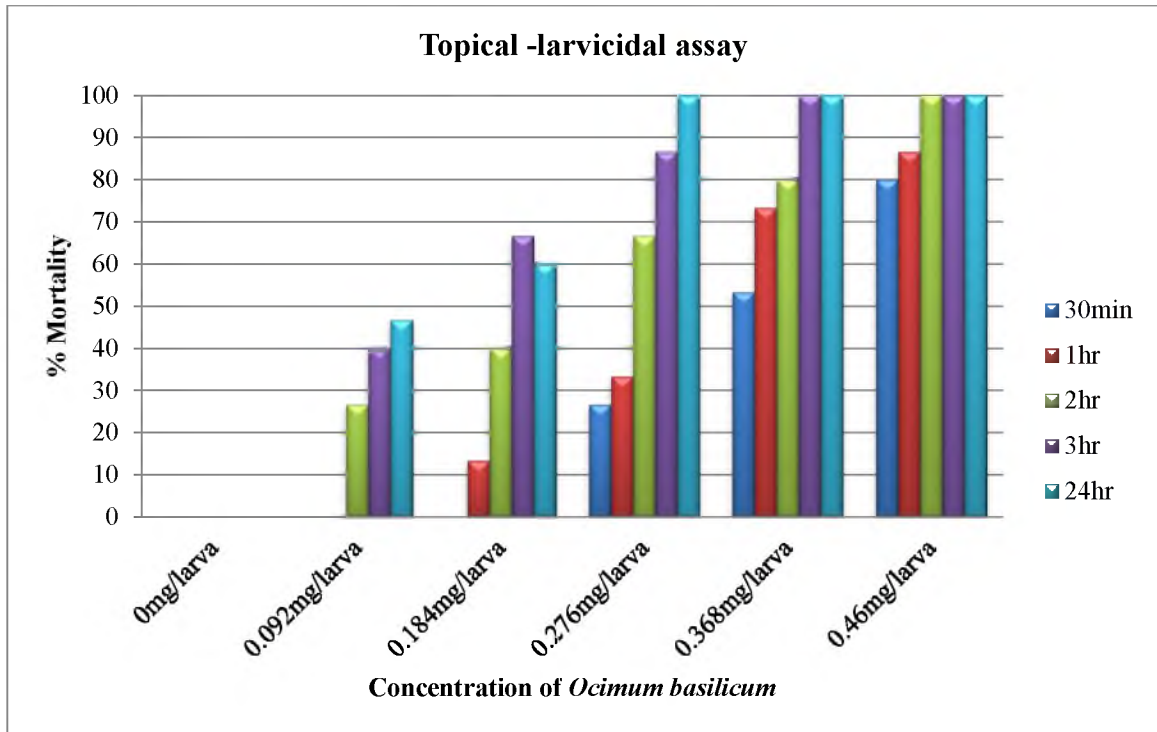


Fig. 111: Contact toxicity of *M. domestica* larvae with *O. basilicum*

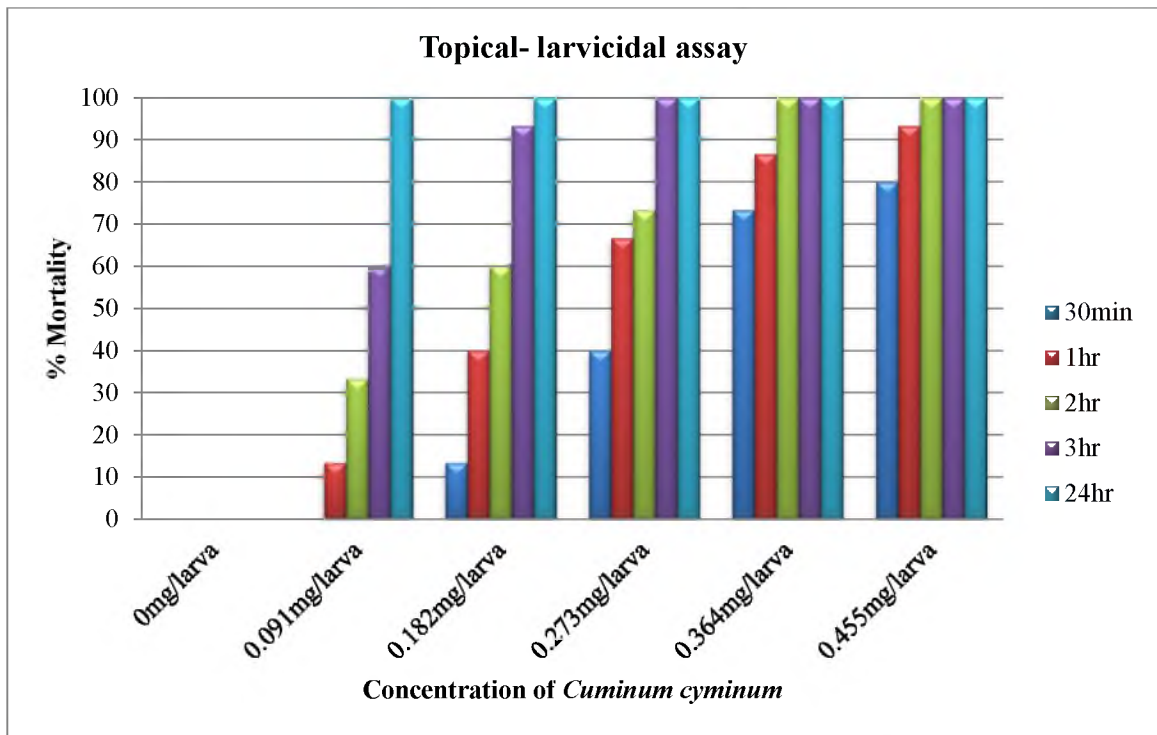


Fig. 112: Contact toxicity of *M. domestica* larvae with *C. cyminum*

Table 15: Percent inhibition of adult emergence with different concentration of basil and cumin seed

Treatment (mg/pupae)	basil	Treatment (mg/pupae)	Cumin seed
0	0	0	0
0.046	26.67	0.0455	13.33
0.092	33.33	0.091	33.33
0.46	60	0.455	53.33
0.92	100	0.91	100

Lethal concentration, LC_{50} and LC_{99} was 0.25mg/cm^3 and 0.98 mg/cm^3 , respectively for basil and 0.30mg/cm^3 and 0.97mg/cm^3 , respectively for cumin seed. The LC values of basil were lower than the cumin seed suggesting that basil was more effective than cumin seed against pupae. Due to lack of pupicidal activity of neem and curcumin in these two essential oils, all pupae emerged into adults in all treated and control groups.

Larval feed toxicity assay

Larvicidal activity of basil, cumin seed essential oil against housefly larvae by inoculating oils into the larval feed was found to be dose and time dependent. The percent mortality of larvae with feed toxicity assay is depicted in (Fig. 113 and 114). Cumin seed caused more mortality which was found to be more effective against feed toxicity compared to basil. There was no larval feed toxicity of neem and curcumin on larva leading to no mortality in all the larvae resulting in pupation and emergence of adults.

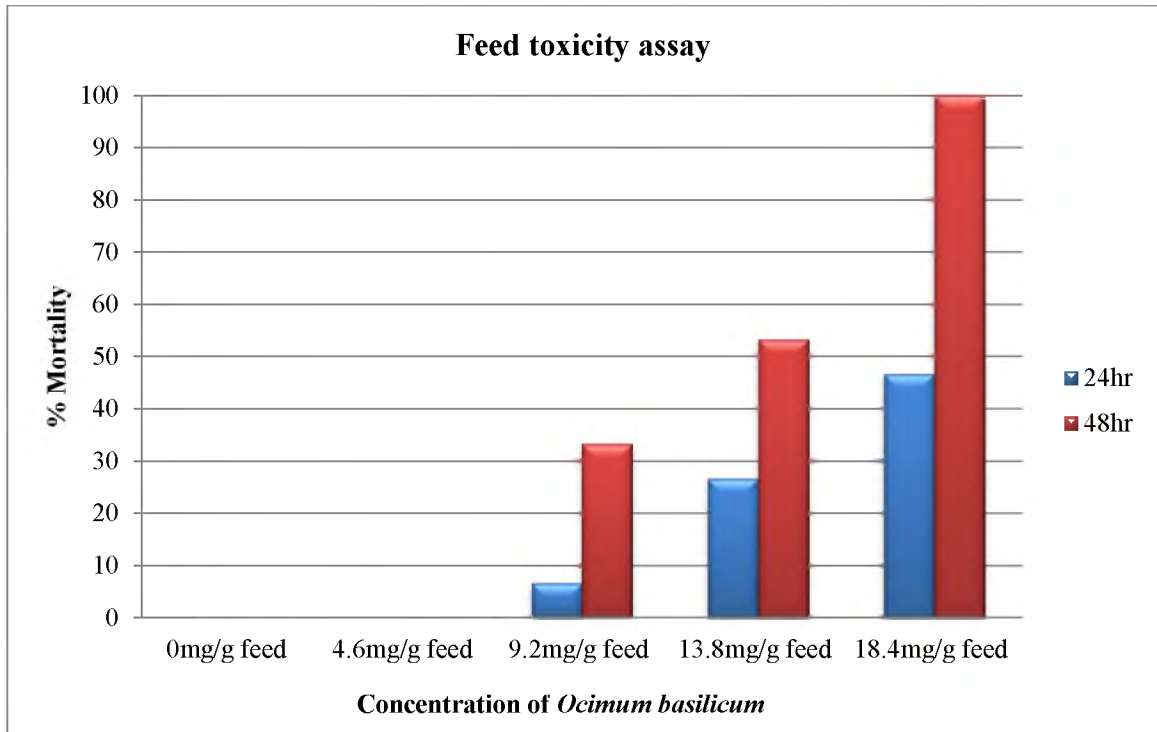


Fig. 113: Feed toxicity of *M. domestica* larvae with *O. basilicum*

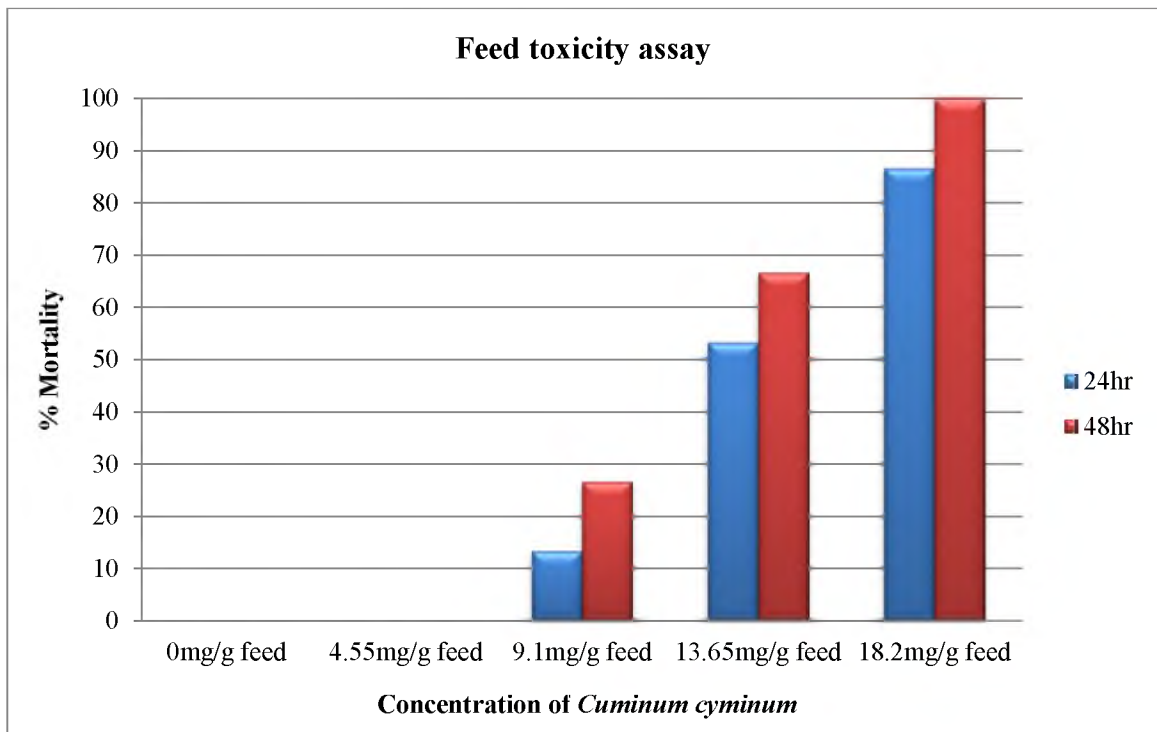


Fig. 114: Feed toxicity of *M. domestica* larvae with *C. cyminum*

Residual film coat bioassay**Adulticidal bioassay**

In residual film coat bioassay, adulticidal activity of basil, cumin seed, neem and curcumin essential oil was found to be dose and time dependent. The percent mortality of adults with residual coat assay is depicted in (Fig. 115, 116 and 117).

Larvicidal bioassay

In the residual film coat the larvicidal activity of basil, cumin seed, neem and curcumin essential oil against housefly larvae was found to be dose and time dependent. The percent mortality of larvae with residual film coat assay given in (Fig. 118, 119, 120).

Repellency test against house fly larva

There was no repellency of housefly larvae with basil, cumin seed at 10 μ l/g of feed where as at 25 μ l/g of feed both oils were found to be repellent and the percent inhibition was found to be 100 at 5, 10, 20, 30min and 1hr. Repellency of neem was not observed in larvae in both control and treated food where as at 30min and above curcumin were found to be repellent. At 30min and 1hr there was 100 percent repellency in case of curcumin (Table 16-35).

Ovipositional deterrent assay

The housefly female flies preferred to lay eggs in control plates than test plates. All essential oils showed oviposition deterrent activity in house fly females. The percent effective repellence of various oils is presented in (Table. 36), where cumin seed showed highest percentage of effective repellency (ER%) followed by basil. Neem and curcumin did not reveal good repellency (Fig. 121, 122).

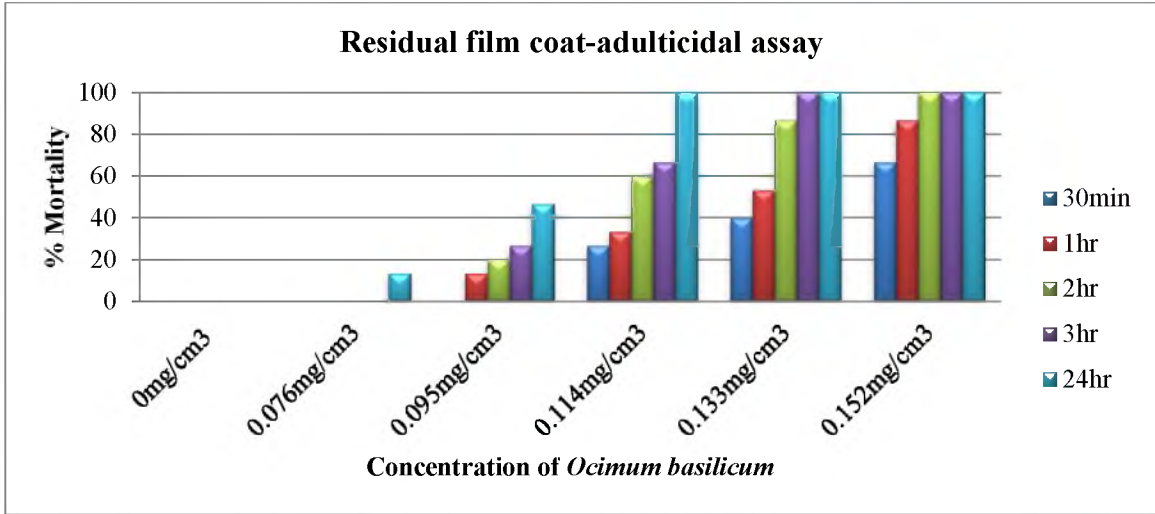


Fig. 115: Residual film coat toxicity of *M. domestica* adults with *O. basilicum*

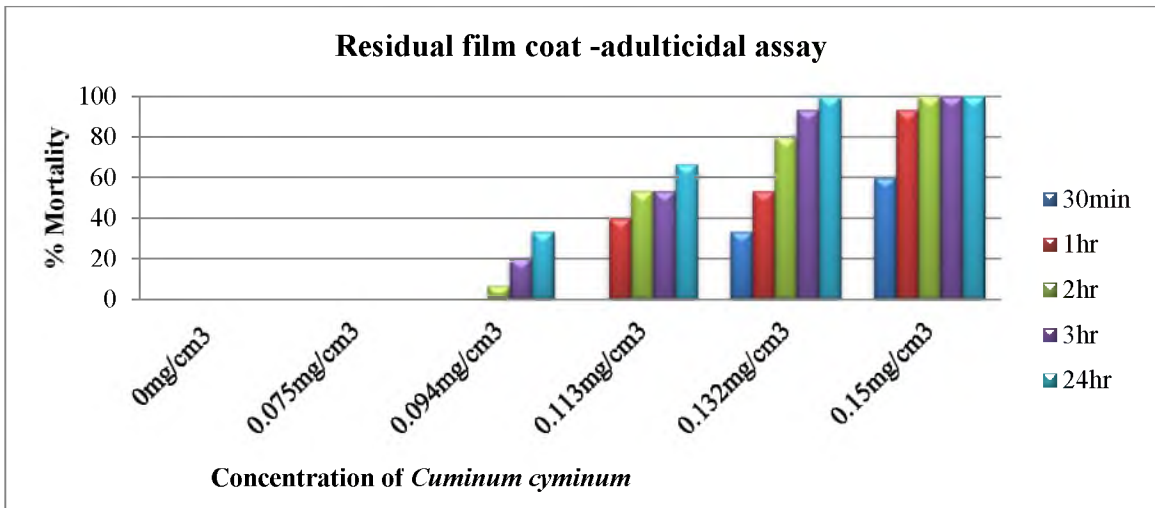


Fig. 116: Residual film coat toxicity of *M. domestica* adults with *C. cyminum*

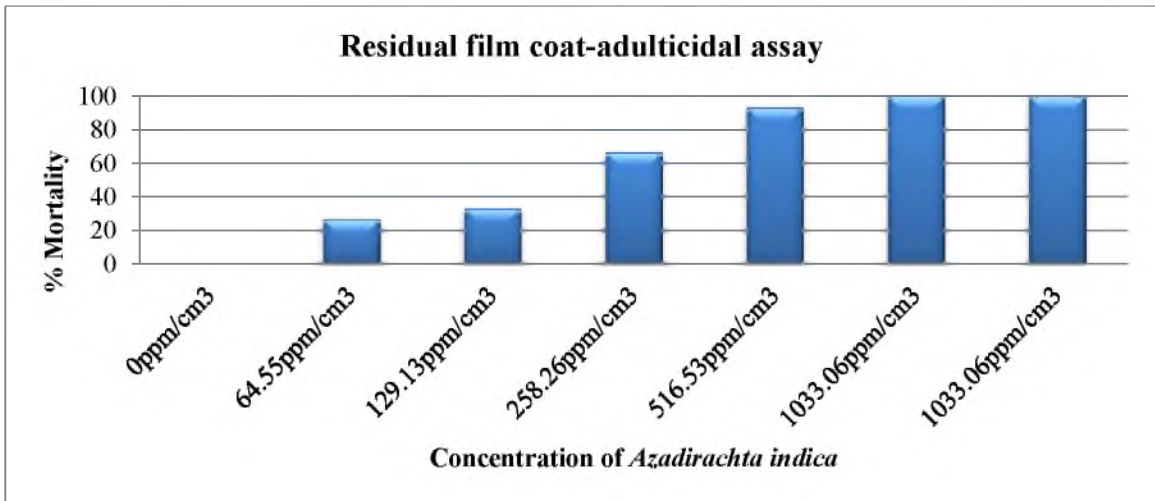


Fig. 117: Residual film coat toxicity of *M. domestica* adults with *A. indica*

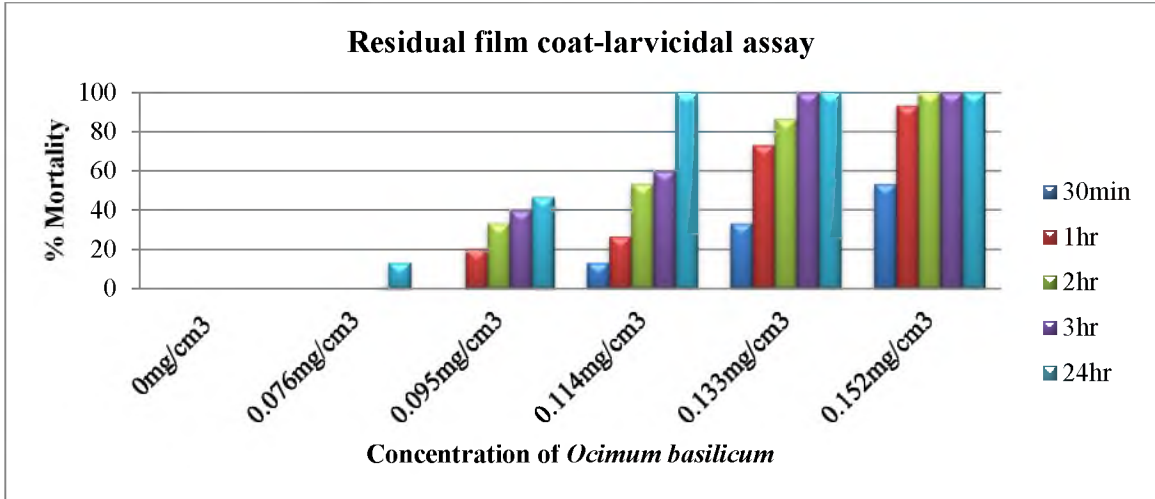


Fig. 118: Residual film coat toxicity of *M. domestica* larvae with *O. basilicum*

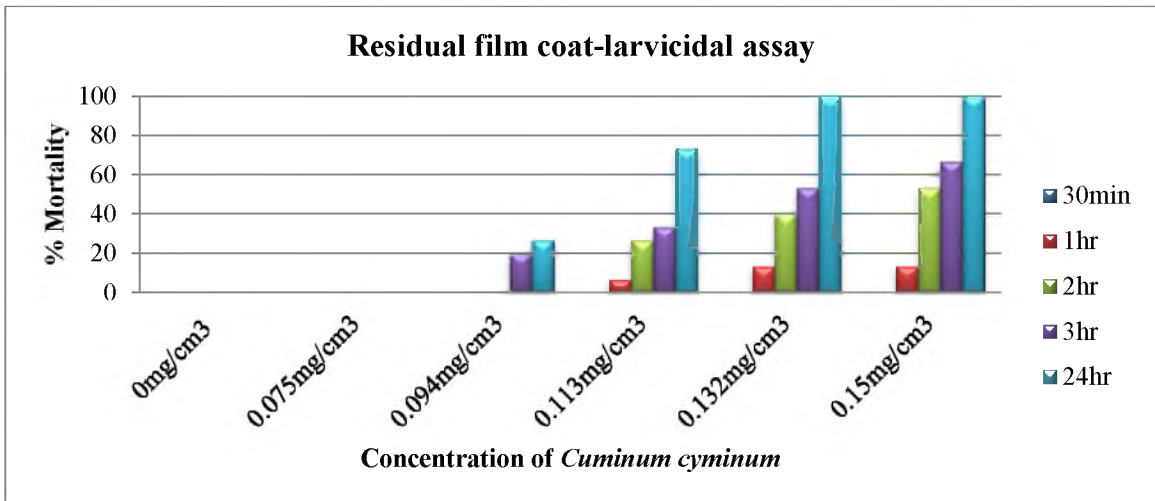


Fig. 119: Residual film coat toxicity of *M. domestica* larvae with *Cuminum cyminum*

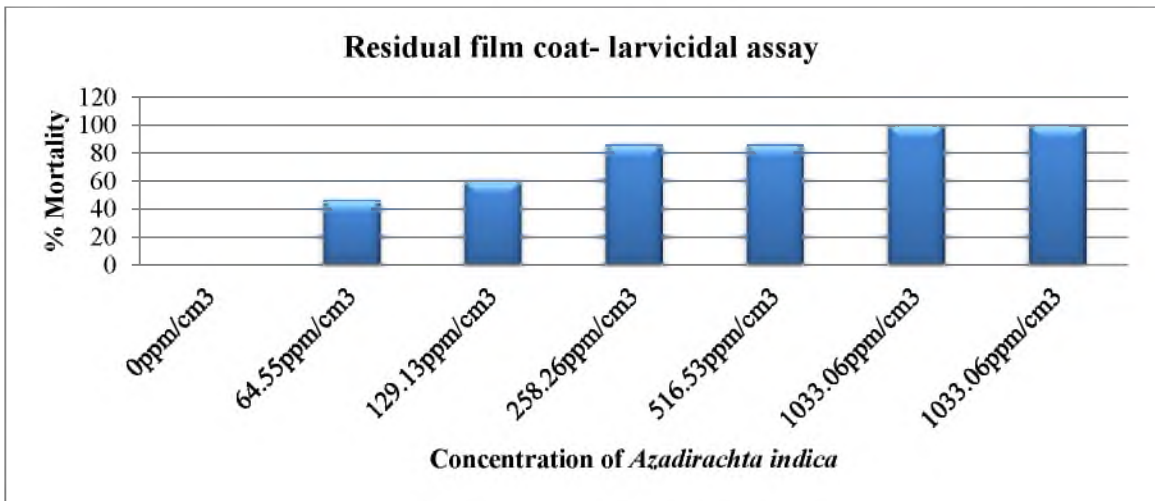


Fig. 120: Residual film coat toxicity of *M. domestica* larvae with *Azadirachta indica*

Table 16: 5 mins repellency – basil

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	2	-20	5	100	2	-20
10 μ l-T	3		0		3	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

PR is percent repellency

Table 17: 10 mins– basil

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	2	-20	4	60	2	-20
10 μ l-T	3		1		3	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 18: 20 mins – basil

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	3	20	3	40	3	20
10 μ l-T	2		1		2	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 19: 30 mins – basil

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	3	20	3	20	2	-20
10 μ l-T	2		2		3	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 20: 1hr– basil

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	4	60	3	20	4	60
10 μ l-T	1		2		1	
25 μ l-C	5	100	5	100	5	100
25T	0		0		0	

Table 21: 5 mins – cumin seed

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	2	-20	2	-20	4	60
10 μ l-T	3		3		1	
25 μ l-C	5	100	5	100	5	100
25T	0		0		0	

Table 22: 10 mins – cumin seed

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	3	20	2	-20	4	60
10 μ l-T	2		3		1	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 23: 20 mins – cumin seed

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	3	20	2	-20	5	100
10 μ l-T	2		3		0	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 24: 30 mins – cumin seed

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	2	-20	4	60	3	20
10 μ l-T	3		1		2	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 25: 1hr – cumin seed

TEST	R1	PR	R2	PR	R3	PR
10 μ l-C	4	60	2	-20	5	100
10 μ l-T	1		3		0	
25 μ l-C	5	100	5	100	5	100
25 μ l-T	0		0		0	

Table 26: 5 mins – curcumin

TEST	R1	PR	R2	PR	R3	PR
Acetone-C	2	-20	3	20	2	-20
6250ppm-T	3		2		3	
Acetone-C	0	-100	5	100	3	20
12500ppm-T	5		0		2	
Acetone-C	0	-100	5	100	2	-20
12500ppm-T	5		0		3	
Acetone-C	1	-60	4	60	0	-100
12500ppm-T	4		1		5	

Table 27: 10 mins – curcumin

TEST	R1	PR	R2	PR	R3	PR
Acetone-C	3	20	2	-20	2	-20
6250ppm-T	2		3		3	
Acetone-C	3	20	2	-20	3	20
12500ppm-T	2		3		2	
Acetone-C	1	-60	4	60	2	-20
12500ppm-T	4		1		3	
Acetone-C	1	-60	4	60	2	-20
12500ppm-T	4		1		3	

Table 28: 20 mins – curcumin

TEST	R1	PR	R2	PR	R3	PR
Acetone-C	4	60	1	-60	3	20
6250ppm-T	1		4		2	
Acetone-C	4	60	1	-60	2	-20
12500ppm-T	1		4		3	
Acetone-C	2	-20	3	20	1	-60
12500ppm-T	3		2		4	
Acetone-C	3	20	2	-20	3	20
12500ppm-T	2		3		2	

Table 29: 30 mins – curcumin

TEST	R1	PR	R2	PR	R3	PR
Acetone-C	5	100	5	100	5	100
6250ppm-T	0		0		0	
Acetone-C	5	100	5	100	5	100
12500ppm-T	0		0		0	
Acetone-C	5	100	5	100	5	100
12500ppm-T	0		0		0	
Acetone-C	5	100	5	100	5	100
12500ppm-T	0		0		0	

Table 30: 1hr – curcumin

TEST	R1	PR	R2	PR	R3	PR
Acetone-C	5	100	5	100	5	100
6250ppm-T	0		0		0	
Acetone-C	5	100	5	100	5	100
12500ppm-T	0		0		0	
Acetone-C	5	100	5	100	5	100
12500ppm-T	0		0		0	
Acetone-C	4	60	5	100	5	100
12500ppm-T	1		0		0	

Table 31: 5 mins repellency – neem

TEST	R1	PR	R2	PR	R3	PR
50 μ l-C	2	-20	3	20	3	20
50 μ l-T	3		2		2	
100 μ l-C	3	20	3	20	1	-60
100 μ l-T	2		2		4	

Table. 32: 10 mins repellency – neem

TEST	R1	PR	R2	PR	R3	PR
50 μ l-C	2	-20	2	-20	2	-20
50 μ l-T	3		3		3	
100 μ l-C	3	20	4	60	4	60
100 μ l-T	2		1		1	

Table. 33: 20 mins repellency – neem

TEST	R1	PR	R2	PR	R3	PR
50 μ l-C	3	20	3	20	3	20
50 μ l-T	2		2		2	
100 μ l-C	4	60	3	20	4	60
100 μ l-T	1		2		1	

Table. 34: 30 mins repellency – neem

TEST	R1	PR	R2	PR	R3	PR
50µl-C	3	20	2	-20	2	-20
50 µl-T	2		3		3	
100 µl-C	3	20	4	60	4	60
100 µl-T	2		1		1	

Table. 35: 1hr repellency – neem

TEST	R1	PR	R2	PR	R3	PR
50µl-C	3	20	3	20	2	-20
50 µl-T	2		2		3	
100 µl-C	4	60	3	20	3	20
100 µl-T	1		2		2	

Table. 36: Oviposition deterrent activity against house fly females

Treatment	% Effective Repellency
Basil (184mg)	76.97
Cumin seed (182mg)	84.34
Neem (50000ppm)	32
Curcumin (50000ppm)	14.7

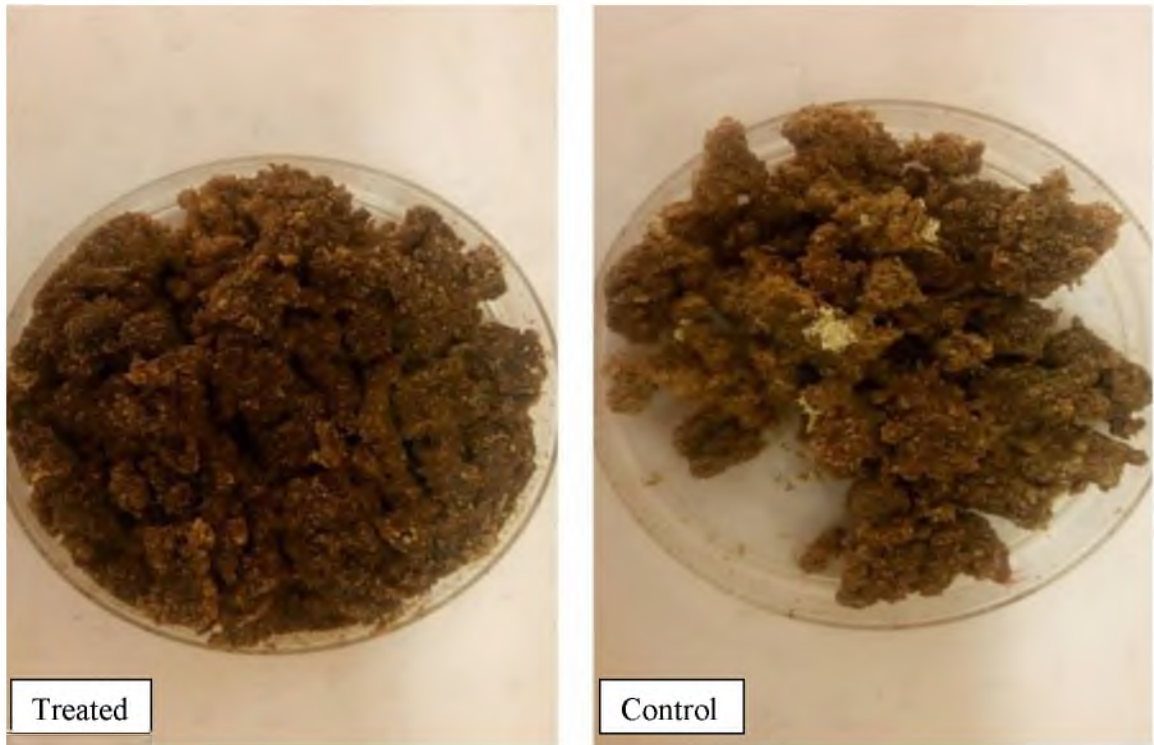


Fig. 121: Ovipositional deterrent assay of cumin seed

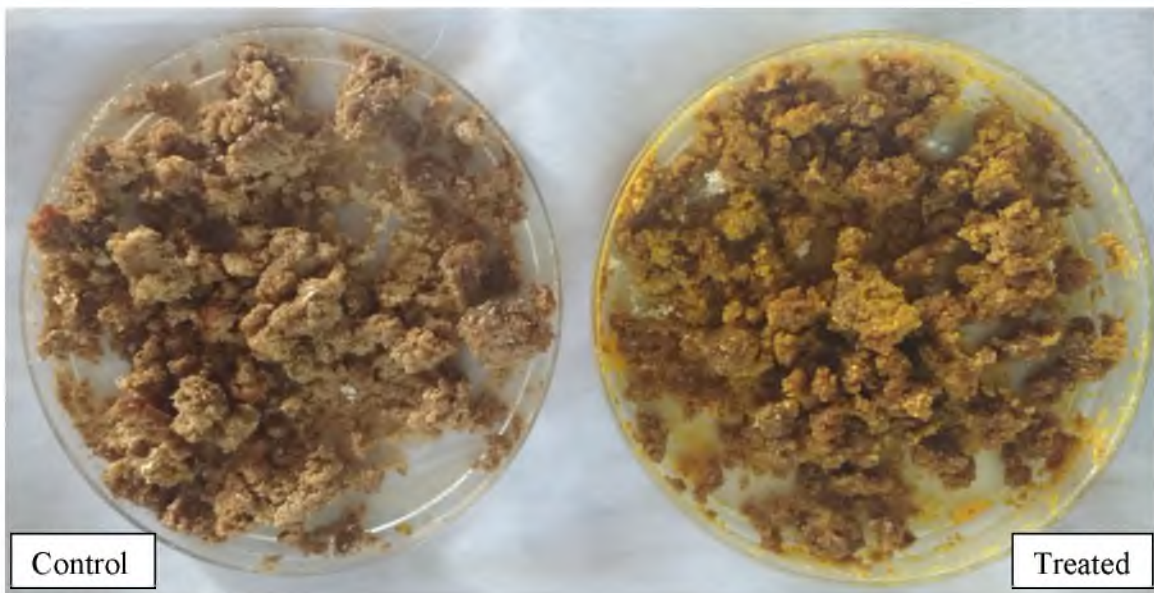


Fig. 122: Ovipositional deterrent assay of curcumin

4.6 Evaluation of new modes of application with semiochemicals

Semiochemicals- “Attraction and kill” strategies, which combines an attractant (semiochemicals) with an insecticide prepared for slow release for control of flies by incorporating in a gel matrix.

Dissipation pattern of gel matrix

The evaporation of water content from the gel matrix was evaluated, where the rate of dissipation upto 4h was minimal where as the dissipation was more at 24hr and onwards at room temperature (35⁰ C and at 40⁰ C). As shown in the (Fig. 123), the rate of dissipation increased as the temperature and time increases. At about 48hr and onwards the water content was almost nil due to evaporation.

Evaluation of different semiochemical baits in laboratory

Different types of semiochemical target baits such as 50ml water+2.5g sugar+50µl of Z-9-tricosene +50µl imidacloprid+0.5g gel matrix, 50ml water+2.5g sugar+50µl imidacloprid+0.5g gel matrix, 50ml water+2.5g sugar+50µl of α-terpeniol+50µl imidacloprid+0.5g gel matrix, 50ml water+2.5g sugar+1g gel matrix were evaluated in the laboratory. The baits were kept in a cage containing 50 adult flies. In three hours of time all the flies were found dead in all semiochemical baits including sugar bait without any attractant on first day of experiment. From next day onwards the dead flies were removed and 50 adult flies were introduced to the same cages containing toxic baits. Upto three consecutive days all the released flies every day died in 6h of time after that there was no death as the gel matrix had dried completely (Fig. 124).

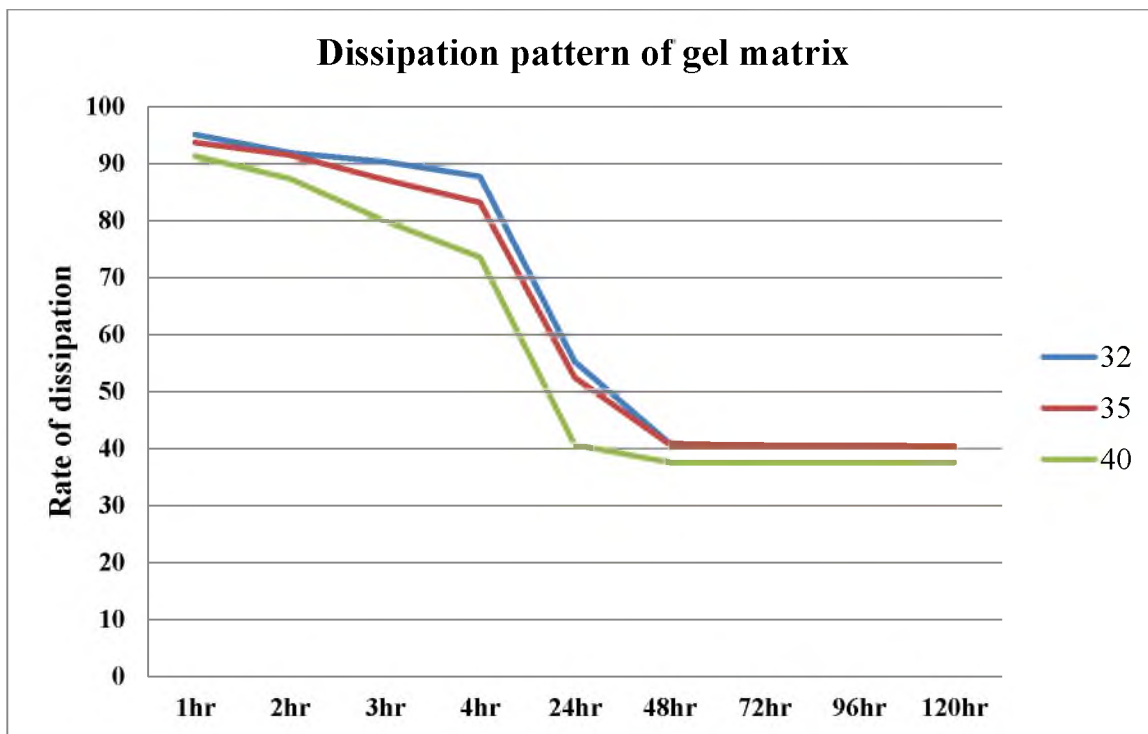


Fig. 123: Rate of dissipation pattern of gel matrix



Fig. 124: Evaluation of semiochemical toxic bait in laboratory

Field assay (in waste disposal management unit): The toxic target baits containing 100ml water+5g sugar+100 μ l of Z-9-tricosene+100 μ l imidacloprid+1g gel matrix (T1), 100ml water+5g sugar+100 μ l imidacloprid+1g gel matrix (T2), 100ml water+5g sugar+20g quick bite+1g gel matrix (T3) (Fig. 126 and 127), 100ml water+5g sugar+100 μ l of α -terpeniol+100 μ l imidacloprid+1g gel matrix (T4) were evaluated in the field for its efficacy of fly attraction and killing. In the present study Z-9-tricosene attracted more flies compared to other attractants as depicted in (Fig. 125). Z-9-tricosene (T1) trapped more flies followed by quick bite (T2), then followed by only insecticide and sugar with gel matrix without any attractant (T3) which was followed by α -terpeniol where it attracted least number of flies (T4). After 48hr, the gel had dried completely and flies were not attracted and killed.

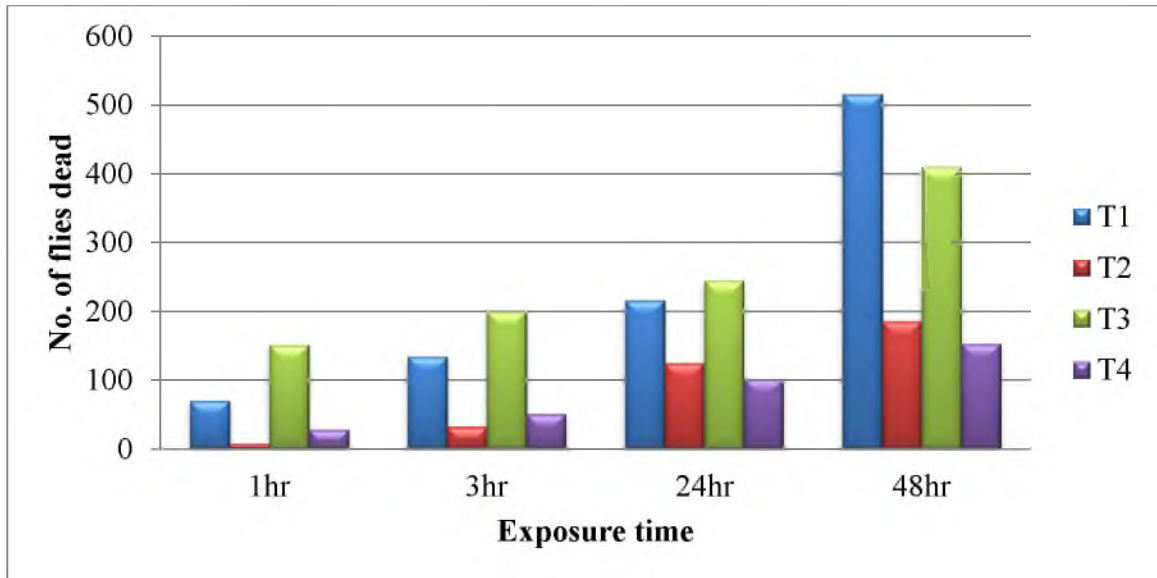


Fig. 125: Evaluation of semiochemical toxic baits in field-number of flies cached



Fig. 126: Evaluation of semiochemical toxic bait in field condition



Fig. 127: Evaluation of semiochemical toxic bait in field condition

4.7 Evaluation of agripower diatomite (diatomaceous earth) for control of flies

Diatomaceous earth application to adult feed

Adults were provided with 2 and 4g DE in 10ml adult food, and were not affected after feeding. The adults lived for 4 days both in control and treated food and later died may be because of insufficient food.

Diatomaceous earth application to artificial diet of larvae

Larvae were provided with 2 and 4g DE in 10g larval food and were not die after feeding. They pupated and emerged into adults.

Diatomaceous earth application to manure of larvae

Manure samples were treated at the concentrations of 2, 4, 8, 10, 20, 40, 60, 80 and 100% of different grades of DE. The larva did not die but pupated and emerged into adults.

Therefore it was concluded that DE was not effective against both adults and larva when given in their diets and natural environment.

Discussion



V. DISCUSSION

Among flies, which were prevalent in poultry farms, housefly *Musca domestica* (Diptera: Muscidae) is a major pest in poultry farms. It represents about 90% of the total fly species associated with poultry manure, especially in caged-layer operations. The housefly is also a major domestic and medical pest. They can cause flyspecking problems by regurgitated faecal material on eggs as well as windows and walls of buildings, equipment, egg trays, lights, egg racks leading to black marks due to their depositions of excreta leading to spoiling of eggs, defacing cages and reduced illumination of lights (Muniyellappa, 2010; Iqbal *et al.*, 2014). High population density of *M. domestica* in poultry farms spoils food and causes annoyance to farm employees. It also causes annoyance to neighbouring rural non-farm communities and often leads to poor community relations and potential litigation. More than 100 pathogens associated with the housefly cause intestinal diseases in humans and animals such as protozoa, bacteria, helminths, fungi, viruses and rickettsial organisms (Barin *et al.*, 2010; Jesus *et al.*, 2004). Accidentally when larvae of *M. domestica* are swallowed in food material they can survive in the human gut and cause intestinal myiasis with symptoms of pain, nausea and vomiting (Shekhawat *et al.*, 1993; Sehgal *et al.*, 2002; Achra *et al.*, 2014). Considering all the aspects of the fly problem in poultry farms with particular reference to houseflies the objectives such as prevalence and diversity of fly infestation in poultry farms, evaluation of new modes of application with semiochemicals, evaluation of biopesticides against fly control, evaluation of biocontrol agents such as entomopathogenic nematodes for fly control, evaluation of agripower diatomite (diatomaceous earth) for control of flies were undertaken in the present study.

5.1 Prevalence and diversity of fly infestation in poultry farms

Morphological identification of adult flies was done by using keys of White *et al.*, 1940; Sabrosky, 1949; James, 1960; Van Emden, 1965; Zumpt, 1965; Fonsseca 1968; Walker, 1994; Triplehorn and Johnson, 2005; Carvalho and Patiu, 2008; Whitworth 2010a and b; Lin *et al.*, 2010; Szpila, 2010; Meiklejohn, 2012; Ramaraj, *et al.* 2014; Irish *et al.* 2014; Akbarzadeh *et al.*, 2015; Oliveira *et al.* 2015.

Morphological identification of larvae were done by using keys of Zumpt, 1965; Holloway 1991; Wells *et al.*, 1999; Sukontason *et al.* 2003a and b; Sukontason *et al.*, 2004a; Brink 2009; Thyssen, 2010; Szpila 2010; Velasquez *et al.* 2010.

In the present study total of six different flies were identified and found to be prevalent in various poultry farms, *viz.* *M. domestica*, *C. megacephala*, *Hy. aenescens*, *Hy. capensis*, *He. illucens* and *S.ruficornis*.

In the present study, the description of morphology made on different adult flies such as *M. domestica* was in accordance with Van Emden, 1965; Zumpt, 1965; Fonsseca 1968; Carvalho and Patiu, 2008. *C. megacephala* was in accordance with White *et al.*, 1940; Zumpt, 1965; Fonsseca 1968; Irish *et al.* 2014; Whitworth 2010a; Ramaraj, *et al.* 2014; Akbarzadeh *et al.*, 2015; Lin *et al.*, 2010; Szpila, 2010; Carvalho and Patiu, 2008. *Hy. aenescens* was in accordance with Sabrosky, 1949; Carvalho and Patiu, 2008. *Hy. capensis* was in accordance with Sabrosky, 1949; Fonsseca 1968. *He. illucens* was in accordance with James, 1960; Carvalho and Patiu, 2008; Oliveira *et al.* 2015 and *S. ruficornis* was in accordance with White *et al.*, 1940; Lopes 1961; Zumpt, 1965; Sukontason *et al.* 2010; Meiklejohn, 2012.

The description of morphology made on different larvae in the present study such as *M. domestica* was in accordance with Zumpt, 1965; Sukontason *et al.*, 2004a. *C. megacephala* was in accordance with Zumpt, 1965; Holloway 1991; Wells *et al.*, 1999; Sukontason *et al.*, 2004a; Velasquez *et al.* 2010; Thyssen, 2010; Szpila 2010 *He. illucens* was in accordance with Velasquez *et al.* 2010; Thyssen, 2010; Oliveira *et al.* 2015.

In different parts of world many authors reported the prevalence of various flies in poultry farms, viz. house fly *M. domestica*, little house fly *Fannia canicularis*, black garbage fly *Ophyra leucostoma*, black blow fly *Phormia regina*, stable fly *Stomoxys calcitrans* and green blow flies *Phaenicia* spp. was reported by Axtell (1970) in United States. *M. domestica*, *S. calcitrans*, *O. chalcogaster*, *F. pusio*, *P. cuprina*, *C. megacephala*, *C. rufifaces*, *Parasarcophaga ruficornis*, *P. argyrostoma*, *Seniorwhitea orientaloidea*, *Volucella obesa*, *Eristalis arvorum* and *He. illucens* were reported by Toyama and Ikeda (1976) in Leeward and Central Oahu. *Simoides pachymera*, *Sepsis lateralis*, *Coproica*, *Fannia leucosticta*, *F. canicularis*, *F. femoralis*, *M. domestica*, *C. putoria* and *M. stabulance* were reported by Hulley (1986) in Grahamstown. Housefly (*M. domestica*), little housefly (*F. annicularis*), dumb flies (*Hy. aenescense*, *Hy. capensis*, *Hy. ignava*), black soldier fly, *He. illucens*, *Drosophila replete*, blow flies such as *Lucilia*, *Phaenicia*, *Phormia*, and *Calliphora*. *L. cuprina* and *L. sericata* was also sometimes associated with poultry farms. Biting flies such as stable fly, *S. calcitrans*, *Culex quinquefasciatus* and *C. pipiens* are also known to occur. Moth flies such as the meal moth, *Pyralis farinalis*, mediterranean flour moth, *Anagasta kuehniella*, and the Indian meal moth *Plodia interpunctella*, cockroaches such as German, *Blattella germanica*, American, *Periplaneta americanum*, and Oriental, *Blatta orientalis* were

reported by Axtell (1999) in USA. *M. domestica*, *F. canicularis*, *Hy. aenescens*, and small dung flies of the family Sphaeroceridae were reported by Rutz (2000) in United States. House fly – *M. domestica*, little house fly – *Fannia* sp. black garbage fly – *Hy. aenescens*, numerous species of blowflies (green or blue bottleflies) and small dungfly – Sphaeroceridae have been reported by Williams (2010) from US. House flies *M. domestica*, little house flies *F. canicularis*, blow flies of Calliphoridae, flesh flies Sarcophagidae, dung flies Sphaeroceridae and fruit flies Drosophilidae were reported by Saif *et al.* (2011). *M. domestica*, *S. calcitrans*, *M. stabulans*, *Fannia* spp, *C. putoria* and *He. illucens* were reported by Tucci (2011) from Brasil. *M. stabulans*, *F. canicularis*, *M. domestica*, soldier fly, *He. illucens*, *S. calcitrans* and *Alphitobius diaperinus*, also known as lesser mealworm were recorded by Levot (2013) from State of New South Wales.

In India, the prevalence of flies in poultry farms were reported by various authors *viz.* housefly (*M. domestica*), little housefly (*F. annicularis*) coastalfly (*F. femoralis*), latrine fly (*F. scalaris*), false stablefly (*M. stabulans*), blow flies, flesh flies, and filth flies were reported by Muniyellappa (2010). Housefly, *Musca domestica* as a major pest species, black garbage fly *Hydrotaea aenescens* and little house fly, *F. canicularis* were reported by Harikrishnan (2009 and 2011a) in Namakkal, Tamil Nadu. Houseflies, other flies with less importance such as *Chrysomya* and *Fannia* were reported by Ponnudurai (2013) in Namakkal, Tamil Nadu.

Among the six species of flies which were recorded in the present study, were also reported by previous workers in poultry farms *viz.* *M. domestica* was reported in all the reports in India and also in other countries representing it as one of the major fly

species. *C. megacephala* was reported by Toyama and Ikeda (1976), Ponnudurai (2013). *Hy. aenescens* was reported by Axtell (1999), Rutz (2000), Williams (2010), Harikrishnan (2011a). *Hy. capensis* reported by Axtell (1999). *He. illucens* reported by Toyama and Ikeda (1976), Axtell (1999), Tucci (2011) and Levot (2013) and *S. ruficornis* was reported by Toyama and Ikeda (1976).

General prevalence of flies as in the present study was reported by various authors in India viz, *M. domestica* by Nandi and Sinha (2004) from Sundarbans biosphere, Bharti (2008 and 2009) from Punjab, Muniyellappa (2010) from Bangalore, Harikrishnan (2009 and 2011a) in Namakkal, Tamil Nadu, Roy *et al* (2012) from West Bengal, Ponnudurai (2013) in Namakkal, Tamil Nadu, Subaharan and Verghese (2014) from Bangalore.

C. megacephala was reported by Nandi (2000) from Sikkim, Singh and Bharat (2000) from Punjab, Bharti (2009), Nandi (2004) in Kolkata, Bharti (2011) from Punjab, Ponnudurai (2013) in Namakkal, Tamil Nadu, Subaharan and Verghese (2014) from Bangalore, Ramaraj *et al.* (2014) from Tamil Nadu and Divya and Sathe (2015) from Western Ghats, Maharashtra, India.

Hy. aenescens was reported by Harikrishnan (2011a) from Namakkal, Tamil Nadu. *Hy. capensis* was reported by Bharti *et al* (2001) from Uttar Pradesh and Bharti (2008) from Punjab. *He. illucens* was reported by Roy *et al* (2012) from West Bengal, Gayatri and Madhuri (2013) from Maharashtra. *S. ruficornis* was reported by Roy and Dasgupta (1977) from West Bengal, Nandi (1992) from Gujarat, Nandi (1993) from Maharashtra, Patil (2013) from Ahmednagar, and Subaharan and Verghese (2014) from Bangalore.

In all the eleven poultry farms in present study, *M. domestica* was found to be prevalent in all the farms and in all the seasons. Whereas other flies such as *C. megacephala* was prevalent in only four farms such as Pragathi Poultry Farm, Nelamangala, Bangalore rural; Venkateshwara Breeding Farm, Kannur, Bangalore East; Monarca Hatcheries, Honnenahalli, Bangalore North and CPF Poultry Farm, Mandur, Bangalore East in rainy season. In summer and winter season in only two farms it was prevalent such as Venkateshwara Breeding Farm, Kannur, Bangalore East, and CPF Poultry Farm, Mandur, Bangalore East, wherein semi-liquid faeces was found throughout the year in these farms making it favourable for the breeding of flies. *Hy. aenescens* was reported in only one farm during the study in rainy season in Venkateshwara Breeding Farm, Kannur, Bangalore East. *Hy. capensis* was observed in only one farm viz. CPF Poultry Farm, Mandur, Bangalore East during the rainy season. *S. ruficornis* was reported in Pragathi Poultry Farm, Nelamangala, Bangalore rural in rainy season and *He. illucens* was found in Dhavangere Poultry Farm, Davangere, in all seasons. The prevalence of flies in poultry farms varied probably because of prevalence of favourable breeding conditions in different farms suitable for breeding of different flies. The prevalence of *C. megacephala* was more in rainy season and was previously reported by Valandikar and Hiregoudar (1996). Ponnudurai and Harikrishnan (2011) also reported that the high fly population in the rainy season and low prevalence in winter but in summer the population increased.

Among different flies, only the larvae of *M. domestica*, *C. megacephala* and *He. illucens* were found to be present in poultry manure whereas larvae of other flies such as *Hy. aenescens*, *Hy. capensis* and *S. ruficornis* were not found in the poultry manure.

Sarcophaga ruficornis flies usually breed in dead carcass and were hence not found in manure.

In case of cage rearing system, all flies were found to be prevalent whereas in deep litter system of rearing only *M. domestica* was prevalent as the heaping of poultry droppings under cages and accumulation of manure with moisture was favourable for breeding of different kinds of flies. In deep litter system of rearing the dry condition of manure and constant disturbance of the deep litter is not favourable for fly breeding.

This constituted the first systematic and extensive study in Bangalore district and no reports are available from other parts of Karnataka state and could not be compared.

5.2 Barcoding of flies

The identification based on various morphological features is tedious and time-consuming. Morphological method needs a specialist, holotype comparison is time consuming and difficult to identify large number of insects in a short period of time, requires taxonomic expertise and morphological similarity in females of certain species complex of insects is a challenge in identification. Therefore, molecular method of identification was advocated. Molecular method of identification was found to be faster and had the advantage of sensitivity and specificity.

DNA barcoding has been widely used in species identification and biodiversity research (Kim *et al.*, 2012) because it has been shown that in many groups, including insects, interspecific variation in DNA sequences of some genes is much higher than intraspecific and this provided an opportunity to use DNA sequences for species

identification. Cytochrome oxidase I (COI) barcoding sequences can be used to discover cryptic, closely related and morphologically similar species. DNA barcoding has gained increased recognition as a molecular tool for species identification in various groups of organisms (Rivera and Currie, 2009) and this provides a reliable, cost-effective and accessible solution to exact species identification (Hebert *et al.*, 2003). In the present study all the flies which were identified morphologically were confirmed by DNA barcoding.

A DNA barcode is a short sequence from standardized portions of the mitochondrial genome (a 648 bp of mtCOI). The concept for barcoding is to study genetic variation between species which exceeds variation within species.

Mt DNA is used for DNA barcoding because Mt DNA is much smaller than nuclear DNA, sequencing is easy. Mt DNA has fast mutation rate with a significant variation between species and less variation among species. It is less prone to insertions, deletions and other large scale of rearrangements which spoils the generation of barcode. It is very stable with little or no degradation in museum specimens and easy to design primers, easy alignment having short segments with less cost to sequence.

COI is used for DNA barcoding as it is the most popular barcode used worldwide and very efficient for species identification. It is highly conserved DNA sequence within species, its alignment process is not difficult- protein coding region and it is easy to isolate from wide range of organisms.

Hebert *et al.* (2003) established that the mitochondrial gene cytochrome *c* oxidase I (COI) could serve as the core of a global bioidentification system for animals. COI gene of animals, insects was amplified by PCR, they yielded specific amplicon of 658bp. In the present study COI of all flies yielded an amplified fragment of 658bp which is in agreement with Hebert *et al.* (2003). The 658 bp of COI sequence of *M. domestica*, *C. megacephala*, *Hy. aenescens*, *Hy. capensis*, *He. illucens* and *S. ruficornis* after BLAST analysis confirmed the species identified based on morphology. In accordance with the present study various authors used COI barcoding by using universal primers by amplifying 658bp length of gene such as Nelson (2008) used COI barcode to identify morphologically identified *Chrysomya* species (*C. flavifrons*, *C. latifrons*, *C. megacephala*, *C. nigripes*, *C. rufifacies*, *C. safranea*, *C. semimetallica*, *C. varipes* and *C. incisuralis*) from East Coast of Australia. Boehm *et al.* (2012) used it for identifying various flies belonging to Calliphoridae and Muscidae in Germany. Bhaskaran and Sebastian (2015) used the method for identifying *Lucilia sericata* from India. Nzelu *et al.* (2015) used it to identify sand flies in Peru. Arnaldos *et al.* (2015) used COI barcoding for identification of *Sarcophaga tibialis* and *Sarcophaga cultellata* from Spain. Meiklejohn *et al.* (2013) for identification of immature life stages of a forensically important fleshfly (Diptera: Sarcophagidae). Archana *et al.* (2014) reported the identification of *C. imicola*, *C. oxystoma*, *C. peregrinus*, *C. anophelis*, *C. palpifer*, *C. huffi*, *C. innocuus* and *C. circumscriptus*. Gutierrez *et al.* (2014) identified sand fly species (Diptera, Psychodidae, Phlebotominae) in Colombia. Rolo *et al.* (2013) used for identifying flies belonging to Calliphoridae and Muscidae in Iberian Peninsula. Pinto *et*

al. (2015a) identified neotropical sand flies (Diptera, Psychodidae, Phlebotominae) in Brazil.

In present study, identification of six different fly species based on morphology was confirmed by DNA barcoding to prove their correct identity. Even the immature stages of the fly *viz.* larvae were identified upto species level by using barcoding as morphological species identification at any life stage is very challenging. Interspecific variation in DNA sequences of COI genes is much higher than intraspecific and this provided an opportunity to use DNA sequences for exact species identification.

5.3 Evaluation of bio control agents such as entomopathogenic nematodes for fly control

Use of EPNs could offer an effective and safer alternative to chemical control (Poinar and Callaghan, 1992). In this regard, Entomopathogenic nematodes (EPN) from the families Steinernematidae and Heterorhabditidae are excellent candidates. They are important biological control agents for a variety of economically important pests (Grewal *et al.*, 2005). They have a mutualistic symbiosis with bacteria in the genera *Xenorhabdus* spp. and *Photorhabdus* spp., respectively. Some researchers suggested that nematodes were effective for controlling fly population in poultry houses (Belton *et al.*, 1987). Where as many researchers indicated that EPNs are not effective for *M. domestica* control in poultry manure (Renn *et al.*, 1985; Geden *et al.*, 1986; Georgis *et al.*, 1987). The survivability and infectivity of EPN can vary as much among conspecific strains as between different species of nematodes (Molyneux *et al.*, 1983) therefore evaluation of additional nematode strains may reveal differences in manure tolerance.

In the present study it was observed that, infective juveniles of *S. feltiae*, *H. indica*, and *S. carpocapsae* showed higher survivability in poultry manure, whereas *S. glasseri* and *S. abassi* were susceptible to manure. After 24h exposure of EPNs to the poultry manure, virulence capacity was 100% but their reproduction capacity decreased presumably due to effect of toxic effect of manure contents on the EPNs. Survivals of all the five EPNs were drastically reduced with increase in exposing time. In poultry manure the presence of live IJs of *S. feltiae*, *H. indica*, and *S. carpocapsae* were recovered up to 96h whereas, *S. glasseri* was recorded up to 72h and *S. abassi* in 24h. Geden *et al.* (1986) reported that *H. heliothidis* juveniles were dead within 1 h of application to poultry manure. These findings showed that the survival of EPNs in poultry manure may be species specific and poultry manure had negative effects on nematode survival. Georgis *et al.* (1987) reported that 100% mortality was observed in *S. feltiae*, *H. heliothidis* and 93% in *S. bibionis* within 24h of exposure of EPNs to the poultry manure. Renn *et al.* (1985) also reported that survivability of *H. heliothidis* and *S. feltiae* was reduced after exposing to the poultry manure in *invitro* studies. Many researchers reported that the decrease in the survival of EPNs in poultry manure may be due to presence of ammonia, salts or other materials associated with the poultry manure which were probably toxic to the EPNs.

Exposure of fly eggs and pupae to EPNs at 5,000 and 10,000 IJs/host did not result in any effect on the egg. The hatching time of eggs is short at 8-12h at 25-35⁰C (Zumpt, 1965) 6-24h (Muniyellappa, 2010) whereas the nematodes require at least 24-72h exposure time for killing of host. In the present study pupae were resistant to all EPNs tested. Similarly, Renn *et al.* (1985) reported that pupae were refractory to the

EPNs but Geden *et al.* (1986) reported that nematodes had entered and parasitized a small number of pupae upon dissection of pupae i.e 1% and 2% in case of *S. feltiae* and *H. heliothidis*, respectively and suggested that these infections may be due to result of physical damage to the puparia during handling before treatment in petri dishes. The housefly pupae were refractory to the parasitism probably due to the formidable barrier presented by the puparium. The pupae of some insects appear to be less susceptible to nematode infection than the larval stages (Kaya and Hara, 1980). The only portal of entry to the insect puparia of *M. domestica* is via the spiracles, but the presence of spiracular slits within these openings may have prevented penetration (Bedding and Molyneux, 1982).

In the present study, the LC₅₀ and LC₉₀ values indicated that second instar larvae were more susceptible to all EPNs compared to third instar larvae. The similar results were reported by Renn *et al.* (1985) and Geden *et al.* (1986). The lower susceptibility of third instar larvae compared with second instar larvae was probably due to the shorter effective exposure period of the larvae and also cuticular changes in the larvae before pupation could have further reduced the successful infection by *H. heliothidis* (Geden *et al.*, 1986). Whereas Mahmoud *et al.* (2007) reported that the third instar were more susceptible compared to second instar larvae of house fly. Larvae were more susceptible to EPNs on filter paper without artificial diet than when provided with artificial diet. In artificial rearing media the mortality was reduced. This may be due to presence of food to the larvae, the larvae develop fast leading to shorter exposure period and also changes in the cuticle leads to reduced infection by EPNs. The moist inner region of the medium

where the larvae concentrated had poor oxygen level which was toxic to juveniles (Geden *et al.*, 1986).

Among the EPNs tested, *H. indica* showed high virulence capacity compared to other EPNs (Steinernematid) used. This is in contrast to the finding of an earlier report where *S. feltiae* was most virulent than *H. heliothidis* (Renn *et al.*, 1985). *Steinernema feltiae* was the most virulent species toward maggots followed by *S. carpocapsae* in the filter paper assay (Taylor *et al.*, 1998). Mullens *et al.* (1987) and Taylor *et al.* (1998) reported that *Heterorhabditis* species had low virulence whereas Geden *et al.* (1986) reported that *H. heliothidis* was the most virulent than *S. feltiae*. Geden *et al.* (1986) reported that larvae were not susceptible to *S. glasseri* in contrast to the present study where larvae were susceptible to *S. glasseri*.

Poultry manure assay revealed that, *H. indica* caused 25% mortality in second instar maggots whereas, *S. carpocapsae* caused only 6.25% mortality at 256000 IJs/larva. Renn *et al.* (1985) reported that no immature stages of *M. domestica* were parasitized after inoculation of *S. feltiae* and *H. heliothidis* at dose of 5000/larva in poultry manure which is in accordance with present study. In contrast to the present study maximum of 28 and 21% mortality was recorded after inoculation of *S. feltiae* and *H. heliothidis* at the dose of 5000/larva. The poor survival and limited movement of nematodes in poultry manure appeared to make them unlikely candidates for biological control of *M. domestica*.

Reproduction and recycling of EPNs in the host play a vital role in their persistence in the environment after application, and thus in overall effectiveness of pest

control (Harlan *et al.* 1971; Georgis and Hague 1981). In this present study, all five EPN species reproduced in third instar of *M. domestica* and their IJs emerged from the host cadavers. However, progeny production assay revealed *H. indica* was significantly greater than that of *S. feltiae*, *S. carpocapsae*, *S. glasseri* or *S. abassi*. Moreover, the present study with progeny production agreed with the reports of Mannion & Jansson (1992); Yadav and Lalramliana (2012) who found that Heterorhabditid nematodes produced significantly more infective juveniles per cadaver than Steinernematid. One possible reason for this difference in EPNs progeny production in third larval stages of *M. domestica* may be related to the type of reproduction of *Heterorhabditids*. *Heterorhabditis indica* is a hermaphrodite that produces more offspring than *Steinernematids*, which are amphimictic (Poinar, 1990; Mannion & Jansson, 1992).

Efficacy of three EPNs *Steinernema carpocapsae*, *S. thermophilum* and *H. indica* were evaluated on engorged females of cattle tick *Rhipicephalus haemaphysaloides*. *H. indica* was highly pathogenic at a concentration of 1250 IJs/ petri dish, inducing 100% mortality within 48-72h when compared to *S. carpocapsae* and *S. thermophilum* whereas *S. carpocapsae* induced 100% mortality in *R. haemaphysaloides*, within 72-96h. *S. thermophilum* induced 100% mortality within 72-144 h and also produced reduction in egg production and decrease in hatchability (Hussain *et al.*, 2016). The ticks were found to be highly susceptible to EPNs. EPNs can be applied to the soil in order to control ticks in environment as they breed in bushes, cracks and crevices of walls and vegetation where its survival will not be affected as the EPNs survive in the soil. Whereas in present study, the fly larvae were highly susceptible to all the five EPN species and all these EPNs successfully reproduced in third instar larvae. *H. indica* showed high virulence

capacity compared to other EPNs (Steinernematid) used. On filter paper assay EPNs proved to be very good biological agents against control of housefly larva whereas the decrease in larval mortality in manure suggests that biocontrol of housefly by using EPNs is unlikely. This may be because of poor survival and limited movement of nematodes in poultry manure which may be due to ammonia or other waste products which are toxic to the EPNs in poultry manure. It may, therefore, be concluded from this study that these EPN isolates are not potential biocontrol agents against house fly *M. domestica* in poultry manure under field conditions.

5.4 Evaluation of new modes of application with semiochemicals

It is well known that chemical insecticides for control of housefly had many disadvantages. Therefore one of the alternate methods for control of housefly was considered with the use of semiochemicals. Toxic bait preparation by using pheromone attractant and insecticide was advantageous over insecticide sprays with greatly reduced exposure to humans and resistance development is less likely if the insecticide is ingested compared with cuticular absorption (Chapman *et al.*, 1998). Semiochemicals are small organic compounds that transmit chemical messages. They are used by insects for intraspecific (pheromones) and interspecies (kairomones and allomones) communication. Insects detect semiochemicals directly from the air with olfactory receptors. In most insects, the receptors are located in sensilla hairs on the antennae. Semiochemicals were already studied in the 1880s. Although their chemistry was not yet understood at that time, people already knew about using female insects to lure males into traps.

Insects communicate by means of scents-pheromones. These are chemicals used for signalling through which they locate and identify their mates. They are natural chemicals emitted in micro quantities in the form of a vapour by virtually all known insects. Each insect species has its own unique signature scent. There are different types of pheromones such as aggregation pheromones: compounds that increase the concentration of insects at the pheromone source. Alarm pheromone-compounds that stimulate insects' escape or defense behaviour. Sex pheromones: compounds that help individuals of the opposite sex to find each other. Trail pheromones-among social insects, compounds used by workers to mark the way to a food source, for example. Marking pheromones-compounds used by insects to mark the boundaries of a territory. Semiochemicals can be used to monitor the size of insect pest populations with survey traps or to lure them into traps and to kill them.

In case of sex pheromone a female insect typically puffs out a thousand millionth of a gram of her signature several times in a minute. Males of her species follow this scent to mate with the female. It follows that if we can identify and then duplicate that scent, we have the means of controlling the males of that species. The principle use of insect pheromones is to attract insects to trap.

Many authors (Hanley *et al.*, 2004) tried semiochemical approach by preparing toxic bait by incorporating pheromone and insecticide and applied paint on method and reported unlikely that (Z)-9-tricosene is sufficiently attractive to houseflies to provide an effective and economic lure in outdoor situations as it attracts only for small period of time as the efficacy will be lost early when applied by paint on method. Therefore in

order to improve the efficacy of pheromone and also in order to reduce the contamination of environment with frequent and heavy application of insecticides in the present study new modes of application of insecticides have been evaluated that is by Semiochemicals-“Attraction and kill” strategies. Combining an attractant (semiochemicals) with an insecticide was tried in the present study with slow and sustained release for control of flies by incorporating in a gel matrix.

In the present study, under laboratory evaluation of semiochemical baits within three hours of time all the flies died in all semiochemical baits including sugar bait without any attractant on first day of start of experiment. Upto three days all the introduced flies died in 6hrs but later had no effect due to drying of gel. In field evaluation of all semiochemical baits Z-9-tricosene attracted and killed more number of flies compared to other attractants. This was in accordance with Chapman *et al.* (1998) who used (Z) -9-Tricosene formulations for control of *M. domestica* in caged-layer poultry units where they found that (Z) -9-Tricosene caught significantly greater numbers of *M. domestica* than control targets. Chapman *et al.* (1999) found that (Z)-9-tricosene impregnated beads caught significantly greater numbers of *M. domestica* than control targets. Hanley *et al.* (2004) used (Z)-9-tricosene baited targets for control of the *Musca domestica* in outdoor situations. Initially more flies were caught but later the catch declined probably because (Z)-9-tricosene is primarily a short-range attractant, and fly populations in outdoor situations are generally distributed over a large area. Thus it is suggested that (Z)-9-tricosene may be of little use in outdoor situations. Pospischil *et al.* (2005) found that attractiveness of the WG 10 formulation was increased by a mixture of Z-9 tricosene and a new fly attractant (LEJ 179) developed by Bayer. Butler *et al.* (2007)

used baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. They found that it attracted more flies compared to control. Butler and Mullens (2010) had used (Z)-9-Tricosene on a Southern California dairy against house flies and reported that significantly more flies (male and female) were collected in traps with (Z)-9-tricosene. Bino Sundar *et al.* (2014) used (Z)-9-Tricosene in attracting and killing house flies in field conditions using a sticky trap baited with sugar and reported a significant difference in the number of flies was observed in Z-9-Tricosene treated traps when compared to control traps without (Z)-9-Tricosene. Bino Sundar *et al.* (2016) used (Z)-9-Tricosene based *M. domestica* lure study on a garbage dump yard using plywood sticky trap baited with fish meal. Significantly more number of *Musca domestica* flies were caught in (Z)-9-Tricosene treated fish meal baited traps compared to those traps without (Z)-9-Tricosene. Previous workers had reported that (Z)-9-Tricosene is attractant to the house flies which was also found in the present study.

(Z)-9-Tricosene is a female sex pheromone which attracts mainly males but both male and female mortality was encountered in baits. This may have occurred initially when males got attracted and died, they simulated that presence of group of flies which attracted female flies also to the bait (Chapman *et al.*, 1999). Controlling the males of particular species by trapping and killing prevented breeding and the population reduced gradually.

Imidacloprid which belongs to the chloronicotinyl class of compounds, is most active when it is ingested and in the present study it was found to be effective in killing houseflies for fly control and also (Z)-9-Tricosene was found to be a good attractant

compared to other attractants thus indicating the effectiveness of (Z)-9-Tricosene as an efficient lure in attracting house flies so that eco friendly pheromone based traps can be used in housefly control strategies as part of integrated pest management strategies. In the present study the gel matrix used for slow dispensing of pheromone and insecticide was also found to be effective for 4 days *in vitro* and 2 days *in vivo*.

Hence it was concluded that tricosene as attractant with imidocloprid as insecticide in the semiochemical method was useful for control of houseflies. This method can be used in field conditions for housefly control in poultry farm.

5.5 Evaluation of agripower diatomite (diatomaceous earth) for control of flies

Diatomaceous earth (DE) is pure amorphous silicon dioxide, made up of fossilised diatoms which acts as an insecticide by absorption of epicuticular lipids and fatty acids, leading to desiccation in arthropods (Jean *et al.*, 2015). DE is basically a lethal dust lined with microscopic razor sharp edges, as insects cross over the powder, the edges cut through the insect's protective exoskeleton and causes the insect to dehydrate from outside in. DE is recognized as a safe, non-toxic substance that has low mammalian toxicity and is considered one of the most effective mineral dusts for the protection of stored products (i.e., grain) from insect pest infestations (Shafiqhi *et al.*, 2014). Diatomaceous earth is highly absorptive amorphous silica that can reduce the moisture in poultry litter making it a less attractive environment for flies to breed in and reduce the suitability for larval development.

In the present study there was no mortality of housefly larvae and adults when given through their feed. The diatomaceous earth added to poultry manure at the different

concentrations did not reduce adult house fly emergence compared to untreated manure which is in accordance with the Shiell (2015) who reported that diatomaceous earth added to duck manure at the concentrations tested (1.9 or 4.7%) did not significantly reduce adult housefly emergence or repel flies from landing compared to untreated manure. In contrast Arthur (2000) used DE against red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) reported mortality after one week showing delayed toxic effects from exposure to diatomaceous earth. Arnaud *et al.* (2005) used different formulations of DE against stored pest *Tribolium castaneum* where they reported that some populations can be satisfactorily controlled with some DE formulations but not with others. This is may be one of the reason for not getting any mortality in the present study probably because the efficacy of DE varied with the formulation on pests. Ziaee *et al.* (2007) used DE against *T. castaneum* where they found young larvae were susceptible compared to old larvae and adults and reported that the reproductive potential was decreased in treated compared to untreated. Athanassiou *et al.* (2007) used DE on stored-product beetle species on wheat and maize. They reported that blending of several DEs together may produce a new DE formulation that is highly effective at low dose rates. Kabir *et al.* (2011) used DE against *T. castaneum* on stored maize, sorghum and wheat. They reported that it is possible to control *T. castaneum* using raw DE. Rajasri *et al.* (2014) used DE against grain moth, *Sitotroga cerealella* in stored rice and reported that DE was effective against *Sitotroga cerealella* upto twelve months of storage of rice with less insect damage compared to deltamethrin and untreated control. Jean *et al.* (2015) used DE against *Sitophilus zeamais* in stored maize where it caused significant reduction of progeny emergence, damaged grains and weight loss, but showed no adverse effect on

the viability of the protected seeds. FossilShield could be of value as green stored grain protectant against *S. zeamais*. Ofuya *et al.* (2015) used DE against *Callosobruchus maculatus* in stored cowpea where 100% mortality was observed in 2DAT. All these findings indicated that DE is effective in controlling stored pests compared to house fly in manure (Shafiqhi *et al.*, 2014).

In the present study diatomaceous earth was not effective against house flies in poultry manure and in artificial diet therefore DE cannot be used for the control of house fly.

5.6 Evaluation of biopesticides against fly control

Botanochemicals that include plant extracts and essential oils derived from the plant matrix are alternative options. Plant essential oils have broad spectrum activity against insects and plant pathogenic fungi ranging from insecticidal, anti feedant, repellent and oviposition deterrent, growth regulatory and anti vector activities (Isman, 2006). It was reported that essential oils possessed fumigant insecticide properties due to the presence of acetyl cholinesterase inhibition and octopaminergic action. Recent investigations indicate that chemical constituents of these oils interfere with the octopaminergic nervous system in insects that could be manipulated for pest control. As this target site is not shared with mammals, most essential oil chemicals are relatively non toxic to mammals and fish in toxicological tests and meet the criteria for reduced risk pesticides (Enan, 2001).

Botanical pesticides are the important alternatives to minimize or replace the use of synthetic pesticides as they possess an array of properties including toxicity to the pest,

repellency, antifeedance, insect growth regulatory activities against pests of agricultural importance (Prakash and Rao, 1989, 1986, 1989; Prakash *et al.*; 2003). The botanical pesticides were used in Indian agriculture operations for over a century to minimize losses caused by pests and diseases (Prakash *et al.*, 2003).

Plant essential oils are one of the major types of botanical pesticides used for insect control. The oils are a major source of highly active and potent metabolites with impacts on insect biology, behaviour and physiology (Isman 2006). Essential oils are also typically available in large quantities at reasonable prices owing to their widespread use as fragrances and food flavors (Isman 2000, 2006). Plant essential oils are most commonly collected by steam distillation and mainly consist of complex blends of monoterpenes, sesquiterpenes and phenols that often readily volatilize (Reverchon 1997). Approximately 1,200 compounds have been identified in essential oils, including terpenes and their corresponding aldehydes, ketones and alcohols; phenylpropanoids, hydrocarbons, esters, oxides and sulphur compounds. Owing to their aromatic properties, several studies have documented the ability of essential oils to repel agriculturally related pests. Botanical pesticides are less expensive and easily available because of their natural occurrence especially some of the botanicals like neem, bel, senwar, pyrethrum, tobacco, karanj, mahua and sweet flag etc., which attained the status of potential pesticides of plant origin to be used in IPM of crop field insects as well as in storage ecosystems (Prakash and Rao, 2003). Plant essential oils and their constituents in relation to contact and fumigant insecticidal actions and repellency have been well demonstrated.

Plant essential oils in general possess low mammalian toxicity thus constitute least or no health hazards and environmental pollution. There is practically no risk of developing pest resistance to these products when used in natural forms. This causes fewer hazards to non-target organisms and pest resistance has not been reported except synthetic pyrethroids. No adverse effect on plant growth, seed viability and cooking quality of the grains.

Essential oils and their constituents demonstrated fumigant and contact toxicities towards insects which besides having direct impact of mortality also have several secondary impacts such as anti-oviposition, repellency and antifeedancy (Moretti *et al.*, 2002; Pavela 2008b). These secondary impacts may play an important role in the overall reduction in the population of insects. Moreover essential oils are relatively non toxic to fishes, birds and mammals (Stroh *et al.*, 1998) and are easily biodegradable (Palacios *et al.*, 2009 a and b) these properties of essential oils render it possible to be used in sensitive areas such as schools, restaurants, hospitals, homes, farms etc (Batish *et al.*, 2008; Palacios *et al.*, 2009 a and b). These botanicals fit perfectly well into housefly management programme due to their safety to non target organisms and the environment. Few studies are at preliminary stage for investigating the effect of botanical pesticides on the housefly. Therefore in the present study some of the essential oils such as Curcumin, neem, Sweet basil and Cumin seed were evaluated against eggs, larvae, pupae and adult stages of the housefly.

Sweet basil

The lethal concentration, LC_{50} and LC_{99} at the exposure period of 2hr was $0.030\text{mg}/\text{cm}^3$ and $0.074\text{ mg}/\text{cm}^3$, respectively for basil for fumigant assay of adult houseflies. Lethal concentration, LC_{50} and LC_{99} at the exposure period 24h was $0.10\text{mg}/\text{cm}^3$ and $0.24\text{ mg}/\text{cm}^3$, respectively for basil for fumigant assay of larva. Pupicidal assay with basil essential oil against housefly pupae resulted in a PIR value between 0% to 100% for basil at different concentrations of oil tested. Lethal concentration, LC_{50} and LC_{99} was $0.07\text{mg}/\text{cm}^3$ and $0.14\text{ mg}/\text{cm}^3$, respectively for basil in fumigant assay of pupa. Fumigation-ovicidal assay with basil essential oil against housefly pupae resulted in a PIR value from 0% to 100% for basil at different concentrations of oil tested.

Lethal concentration, LC_{50} and LC_{99} at the exposure period 2h was $0.046\text{mg}/\text{cm}^3$ and $0.06\text{ mg}/\text{cm}^3$, respectively for basil in topical contact assay of adults. Lethal concentration, LC_{50} and LC_{99} at the exposure period 2h was $0.184\text{mg}/\text{cm}^3$ and $0.50\text{ mg}/\text{cm}^3$, respectively for basil in topical contact assay of larva. Pupicidal assay with basil, cumin seed essential oil against housefly pupae resulted in a PIR value between 0% to 100% for basil at different concentrations of oil tested in topical contact assay. Lethal concentration, LC_{50} and LC_{99} was $0.25\text{mg}/\text{cm}^3$ and $0.98\text{ mg}/\text{cm}^3$, respectively for basil in topical contact assay of pupa. In feed toxicity assay of larva 100% mortality was reported in 48h for basil at $18.4\text{mg}/\text{g}$ of feed. In residual film coat toxicity assay of basil against adult and larva 100% mortality occurred at concentration of $0.114\text{mg}/\text{cm}^3$ and $0.132\text{ mg}/\text{cm}^3$ respectively. The repellency of housefly larvae with basil at $25\text{ }\mu\text{l}$ (23mg basil and 22.75mg cumin seed)/g of feed for both oils was 100% at 5min to 1hr. Basil showed

oviposition deterrent activity against house fly females with effective repellency (ER%) of 76.97. The study indicated that sweet basil was very effective against all stages of house fly with low concentration of oil.

This was in accordance to various authors with sweet basil with other pests but on housefly a few studies were conducted and hence the present study is compared with other important pests. Buentello-Wong *et al.* (2016) reported the toxicity of *Ocimum basilicum* against adults of the Mexican fruit fly, *Anastrepha ludens*. At the highest concentration 2.0 % w/v, 100% mortality was observed. Kiplangat *et al* (2013) reported the repellent activity of *O. basilicum* on rabbit skin against *Aedes aegypti* at 3% of oil 100% repellence was observed. Karamaouna *et al.* (2013) reported insecticidal activity of *O. basilicum* against vine mealybug, *Planococcus ficus*. The LC₅₀ values for basil oil ranged from 44.1 to 46.8 mg/mL. Chang *et al.* (2009) reported that trans-anethole, estragol, and linalool are major bioactive compounds in basil oil against tephritid fruit flies. Methyl eugenol is a minor component in basil oil, but it is very potent to *C. capitata* and *B. cucurbitae*. Kerdchoechuen *et al.* (2010) reported the biocontrol of the maize weevil with sweet basil oil which induced a relatively high mortality rate (greater than 90%) at a low volume of 30 ml against the maize weevil on 40 g of rice grain on the first day of exposure and also completely suppressed insect progeny production thereafter. Murugan *et al.* (2007) reported the larval toxicity and smoke repellent potential of *O. basilicum* at different concentrations against the different instar larvae and pupae of *Aedes aegypti*. The LC₅₀ values of *O. basilicum* for I instar larvae was 3.734, II instar 4.154, III instar 4.664 and IV instar 5.124. The LC₅₀ and LC₉₀ of pupae were 16.925% and 15.474% and showed repellent action also. Pavela (2008a) reported insecticidal

property of *O. basilicum* against the *M. domestica* where lethal dose was found to be 15 µg/fly. Aarthi and Murugan (2010) reported the larvicidal and repellent activity of *Ocimum basilicum* against the malarial vector, *Anopheles stephensi*. Bobi *et al.* (2015) reported insecticidal efficacy of ethanol extract of leaf of *O. basilicum* by dipping and feeding method at 100-400ppm. It affected the pupation and emergence of adults. Manzoor *et al.* (2013) reported acaricidal activity of essential oils from Tulsi (*O. basilicum*). The highest acaricidal activity (100 % mortality) was observed with *O. basilicum* oil at 1:15 (66.6 µL/mL). Mohamed (2015) reported the toxicity of basil *viz.* *O. basilicum* extracts on *T. confusum* and found that the last larval instar was more highly susceptible than the adult stages. The LC₅₀ of *O. basilicum* extract were 148.38 and 218.78 ppm for the last larval and adult stages after 24h exposure, respectively. Ahmed *et al.* (2013) reported *O. basilicum* was repellent for oviposition where least number of eggs of 92.56 eggs was laid per female⁻¹ in house fly. Ojianwuna *et al.* (2011) reported the toxicity of *Ocimum suave* (wild basil) leaf oil on adult houseflies. The oil applied caused significant mortality of the housefly. The LC₅₀ was 0.09 mL/50 mL of water and the LT₅₀ was 4.40 h. Fabbro and Nazzi (2008) tested the repellency of sweet basil (*O. basilicum*) against the tick *Ixodes ricinus* by using a laboratory bioassay, eugenol from *O. basilicum* appeared to be repellent. Velazquez *et al.* (2011) reported acaricidal effect of *O. basilicum* against *Rhipicephalus (Boophilus) microplus* which produced 100% mortality at all concentrations with exception of a dramatic decrease at 1.25% concentration. Eliopoulos *et al.* (2015) reported the fumigant activity of essential oil vapors distilled from sweet basil *O. basilicum* against two major stored product pests *viz.* *E. kuehniella*

and *P. interpunctella*. They reported that the oil was highly effective against adults but was less effective against immature stages (larva and pupa).

The monoterpenoids linalool, 1,8 cineole were the predominant components in basil which consisted 62.6% of the essential oil. Monoterpenoids have been considered the most important insecticidal constituents of plant essential oils with key role in fumigant action because of their high volatility. It is well-known that for fumigants, the active stages of insects are more susceptible than the sedentary stages because of differences in their respiratory rate (Eliopoulos *et al.*, 2015)

Ocimum basilicum extracts showed considerable, ovicidal, larvicidal, pupicidal and adulticidal properties, which could be due to the active compound. The higher mortality may be due to the combined action of plant compounds that might be acting on the midgut epithelial cells and exerted their toxic effects. An insect repellent is a chemical acting in the vapour phase prevents an insect from reaching a target to which it would otherwise be attracted (Murugan *et al.*, 2007). The exact mode of action of sweet basil oil is still unknown and its activity is most likely due to camphor and limonene known for repellent activity (Kiplangat *et al.*, 2013). Basil consisting mainly of linalool, methyl chavicol and eucalyptol on several species reported these EOs as highly toxic (Karamaouna *et al.*, 2013).

In the present study significant activity of sweet basil essential oil against eggs, larvae, pupae and adults of house fly paved the way for its uses as eco friendly house fly control measures. Being a commonly available plant it is a potential source of biopesticide for housefly control.

Cumin seed

Lethal concentration, LC_{50} and LC_{99} at the exposure period of 2h was $0.018\text{mg}/\text{cm}^3$ and $0.057\text{ mg}/\text{cm}^3$, respectively for cumin seed for fumigant assay of adults. Lethal concentration, LC_{50} and LC_{99} at the exposure period 24h was $0.08\text{mg}/\text{cm}^3$ and $0.14\text{ mg}/\text{cm}^3$, respectively for cumin seed for fumigant assay of larva. Pupicidal assay with cumin seed essential oil against housefly pupae resulted in a PIR value between 0% to 66.67% for cumin seed at different concentrations of oil tested. Lethal concentration, LC_{50} and LC_{99} was $0.11\text{mg}/\text{cm}^3$ and $0.20\text{mg}/\text{cm}^3$, respectively for cumin seed for fumigant assay of pupa. Fumigation-ovicidal assay with cumin seed essential oil against housefly pupae resulted in a PIR value from 0% to 73.33% for cumin seed at different concentrations of oil tested.

Lethal concentration, LC_{50} and LC_{99} at the exposure period of 2h was $0.059\text{mg}/\text{cm}^3$ and $0.09\text{ mg}/\text{cm}^3$, respectively for cumin seed in topical contact assay of adults. Lethal concentration, LC_{50} and LC_{99} at the exposure period 2h was $0.137\text{mg}/\text{cm}^3$ and $0.39\text{ mg}/\text{cm}^3$, respectively for cumin seed in topical contact assay of larva. Pupicidal assay with basil, cumin seed essential oil against housefly pupae resulted in a PIR value from 0% to 100% for cumin seed at different concentrations of oil tested in topical contact assay. Lethal concentration, LC_{50} and LC_{99} was $0.30\text{mg}/\text{cm}^3$ and $0.97\text{mg}/\text{cm}^3$, respectively for cumin seed topical contact assay of pupa.

In feed toxicity assay of larva 100% mortality occurred in 48h for cumin seed at $18.2\text{mg}/\text{g}$ of feed. In residual film coat toxicity assay of cumin seed against adult and

larva recorded 100% mortality at a concentration of 0.114mg/ cm³ and 0.132 mg/cm³, respectively.

The repellency of housefly larvae with cumin seed at 25 µl (23mg basil and 22.75mg cumin seed)/g of feed both oils was observed and the 100% inhibition was found at 5min to 1hr. Cumin seed showed oviposition deterrent activity against house fly females with effective repellency (ER%) of 84.34.

The effect of cumin seed on housefly was not reported earlier and therefore the present work was compared with other important pests. Chaubey (2008) reported fumigant toxicity of *C. cyminum* against pulse beetle, *C. chinensis*. The essential oils caused death of adults and larvae when fumigated and also reduced the oviposition potential, egg hatching rate, pupal formation and emergence of adults from pupa when fumigated. They reported that toxic and inhibitory effects may be due to suffocation and inhibition of various biosynthetic processes of the insects at different developmental stages. Velazquez *et al.* (2011) reported the acaricidal effect of *C. cyminum* against the cattle tick *Rhipicephalus (Boophilus) microplus*. Cumin, produced 100% mortality in all tested concentrations on *R. microplus* larvae. Kamaraj *et al.* (2011) evaluated the larvicidal and repellent activity of *Cuminum cyminum* and reported 100% mortality.

Major components of *C. cyminum* for all its insecticidal effects were due to cuminaldehyde, γ-terpinene and 2-carene-10-al (Velazquez *et al.*, 2011)

As an insecticide, the mode of action of essential oils or their constituents is not clear. Essential oils affect physiology of insects in diverse ways. The plant essential oils

are lipophilic in nature which facilitates them to interfere with the basic biochemical, metabolic, physiological and behavioural functions of insects. It affects biochemical processes and specifically disrupts the endocrinologic balance in insect body. Activity of essential oils is attributed to their major components and the resulting antagonistic or synergistic action of different components. However, minor components may also contribute to the biocidal activity (Manzoor *et al.*, 2013).

The extracts tested have shown potential toxicidal activity, the active constituent of all the plant species may be contact stomach poison or neurotoxic actions against the various developmental stages of *M. domestica*. All the plant materials screened hindered larval-pupal transformation and adult emergence. This decrease in the adult emergence could be due to the fact that the extracts block the maturation of imaginal discs which are the primordial of many adult integumentary structures in endopterygote insects or due to deformation of adult chitin or may be due to the effects of some active ingredients present in the extracts which exhibit potential to cause interference into the normal metabolism of the insects. The active constituents may transform the alcohol present into the insect body to the corresponding esters and causes alteration in the normal metabolism of the insects which may result to failure in pupation and adult emergence (Bobi *et al.*, 2015).

In the present study significant activity of cumin seed essential oil against eggs, larvae, pupae and adults of house fly pave the way for its use as eco friendly housefly control measure. However the cost effectiveness needs to be considered. Cumin seeds

being a widely used spice is generally costly for human use and may be considered when available in large quantities and in affordable cost.

Neem

In the present study, neem was not found effective against housefly egg, larva, pupa and adult stages when subjected to fumigant toxicity, topical contact toxicity and feed toxicity even at 50000ppm. Whereas, in residual film toxicity, 100% mortality of larva and adult was found at 25000 and 50000 ppm which was in contrast to the observation of Mansour *et al.* (2012) who reported that *A. indica* showed 100% mortality at 1000ppm in topical contact assay. Khan and Ahmed (1998) studied the effect of crude neem leaf extract against housefly where they found LD₅₀ and LD₉₀ values were 8.4 and 169.8 ug/fly of neem extract, respectively. Alouani *et al.* (2009) reported the effect of neem tree extract (azadirachtin) against mosquito larvae and found that it was larvicidal and adult fecundity was markedly decreased and sterility was increased by the Azadirachtin after treatment of the fourth instar and pupal stage. Siriwattananarungsee *et al.* (2008) reported that the effect of neem, *Azadirachta indica*, on ultrastructural alteration of larvae and puparia of the *M. domestica* exhibited swelling of the integument and bleb formation on the integument. Deleito *et al.* (2008) reported that the reduction of housefly emergence from treated pupae with 0.6 per cent of neem oil was 95.6 per cent under laboratory conditions and 94.5 per cent when applied on the soil. Okumu *et al.* (2007) reported the larvicidal effects of a neem (*A. indica*) oil formulation (azadirachtin content of 0.03% w/v) on the malaria vector *A. gambiae*. Neem oil had an LC₅₀ value of 11 ppm after 8 days, which was nearly five times more toxic than the corn oil formulation. Adult emergence was inhibited by 50% at a concentration of 6 ppm. Significant reduction on

growth indices and pupation, besides prolonged larval periods were observed at neem oil concentrations above 8 ppm. Shanmugasundaram *et al.* (2008) reported neem (*A. indica*) and karanja (*Pongamia glabra*) oil cakes against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* where the LC₉₅ for combination of two oil cakes was 0.93, 0.54 and 0.77%, respectively. Dua *et al.* (2009) reported the efficacy of neem oil formulation against 3rd and 4th larval instars of *Anopheles stephensi*, *Culex quinquefasciatus* and *Ae. aegypti*. It was found that LC₅₀ against *An. stephensi*, *C. quinquefasciatus* and *Ae. aegypti* was 1.6, 1.8 and 1.7 ppm, respectively. The neem formulation at 140 mg A.I. /m² was applied in different mosquito breeding sites under field conditions. It was found that the maximum reduction rate of *Anopheles*, *Culex* and *Aedes* was 100%, 95.5% and 100%, respectively. Kiplangat *et al.* (2013) reported the repellent activity of *Azadirachta indica* on rabbit skin against *Aedes aegypti*. Neem oil did not provide complete protection. The mean percent repellency of 5% Neem oil was 84.21. Bobi *et al.* (2015) reported insecticidal efficacy of ethanol extract of leaf of *A. indica* against *M. domestica* which affected the pupation and emergence of adults. Abiy *et al.* (2015) reported repellent efficacy of *A. indica* against *A. arabiensi* complete protection time for neem was 3 hrs.

In the present study, neem was effective only at high dose of 50000 ppm in residual contact assay on larvae and adult and was not found to be effective against eggs, larvae, pupae and adults of house fly in fumigant and contact assay. This might be due to the fact that vapours of neem did not have any suffocation and inhibition of various biosynthetic processes of the insects at different developmental stages. Therefore it is not a good biopesticide for house fly control measures.

Curcumin

In present study, there was no mortality of housefly egg, larva, pupa and adult when subjected to fumigant toxicity, topical contact toxicity, residual film toxicity and feed toxicity even at 100000ppm with curcumin which is in contrast to Pawar (2013) who reported that the mortality of housefly larvae occurred at 115.821ppm and that enzyme activities were decreased. Genetic factors, harvesting time, individual plants, soil type, fertilization, time of collection, mode of drying the plant material, storage period and environmental factors affected the chemical composition and the content of essential oils from *C. longa* rhizomes (Tavares *et al.* 2013). This might be the reason for the present study where curcumin was not found to be effective.

In the present study, there was no ovipositional deterrence activity of curcumin on female house flies. With larvae, at 30 and 60min repellency was observed. In contrast to present study, Tawatsin *et al.* (2006) reported that oviposition deterrent activity of *C. longa* against *Ae. aegypti* was 94.7%, whereas DEET and IR3535 had no repellency. The results revealed that *C. longa* essential oil was an efficient mosquito repellent and oviposition deterrent.

Tavares *et al.* (2013) reported the effect of Ar-turmerone from rhizomes and effects on Maize weevil *Sitophilus zeamais* and fall armyworm *Spodoptera frugiperda*. The ar-turmerone was highly toxic to these pests at low doses with significant repellent action against *S. zeamais*. Ali *et al.* (2014) reported the effect of *Curcuma longa* on *T. castaneum* a stored pest of wheat grain. It caused significant mortality and reduced the larval, pupal and adult emergence.

In the present study, curcumin was not effective against eggs, larvae, pupae and adults of house fly and could not be regarded as a biopesticide for house fly control.

In the present study, it was concluded that the housefly *Musca domestica* was highly prevalent in all the poultry farms as a predominant species. DNA barcoding confirmed all morphologically identified fly species. EPN isolates are not potential biocontrol agents against house fly *M. domestica* in poultry manure because of ammonia and other substances in manure. (Z)-9 Tricosene was found to be good attractant compared to other attractants thus indicating the effectiveness of (Z)-9 Tricosene as an efficient lure in attracting house flies. Semiochemical method with the use of tricosene along with imidacloprid was a effective method of housefly control and recommended for use in poultry farms. Diatomaceous earth was not effective against house fly control. Among the four different essential oils used against different developmental stages of housefly, sweet basil and cumin seed were found to be very effective against house fly control. As the sweet basil is cheaper and readily available compared to cumin seed, it is recommended that sweet basil can be used as a potential biopesticide for control of house flies.

Future perspectives

In conclusion, the use of essential oils in the control of flies is an exciting area which holds huge potential for the future. However, research into the use of essential oils as control agents is still at a preliminary stage. Extensive field trials, standardization of components, standardization of extraction techniques and standardization of experimental design, mammalian toxicology profiling and excipient development, as well as further

investigation into the residual activities and length of shelf-life of these oils are required before their potential can be fully explored.

Further research on blends of essential oils and improved formulations and delivery systems could lead to substantial improvements in the performance of botanicals for vector and sanitary insect control. Further, experiments are needed to evaluate its economic aspects and activity under field conditions. Applied plant oils, like all chemically based medicinal herbal plants, should also undergo a battery of experimental procedures to determine their total pharmacological profile. Enhancement of the potency of oils by adding synergists and stabilisers will prolong their effectiveness.

Further studies on synergistic combinations and isolation of bioactive fraction/constituent may provide futuristic lead products for field application of fly control. The development of a technology to incorporate essential oils into food bait is required to provide a controlled release with sufficient stability, which could then provide an alternative method for the control of flies.

As the reproduction of infective juveniles of entomopathogenic nematodes in house fly larvae was less compared to *Galleria mellonella*. Passaging of IJs in house fly larvae for more generations may possibly lead to adaptability and reproduction in *Musca* also as in the case of *Galleria*.

Summary



VI. SUMMARY

The present study was conducted to know the seasonal prevalence and diversity of fly infestation in poultry farms of Bangalore districts by morphological and molecular methods of identification. Different managerial aspects to control houseflies in poultry farms were evaluated including semiochemicals, biopesticides, entomopathogenic nematodes and agripower diatomite (diatomaceous earth).

In the present study, total of six different flies were found to be prevalent, viz. *Musca domestica*, *Chrysomya megacephala*, *Hydrotaea aenescens*, *Hydrotaea capensis*, *Hermetia illucens* and *Sarcophaga ruficornis*. In the rainy season all six different flies were prevalent whereas in winter and summer *M. domestica*, *C. megacephala* and *He. illucens* were prevalent. Among these flies, larvae of *M. domestica*, *C. megacephala* and *He. illucens* were found to be present in poultry manure. In deep litter system *M. domestica* was prevalent and in case of cage system *M. domestica*, *C. megacephala*, *H. aenescens*, *H. capensis*, *He. illucens* and *S. ruficornis* were prevalent.

The morphologically identified flies in the present study were confirmed by amplification of COI gene using universal primers LCO1490/HCO2198 which yielded specific amplicon of 658bp. Sequences of these species after BLAST analysis confirmed the species which were identified based on morphology. Sequence of these species submitted to GenBank and barcode was generated using BoldSystems v3. The molecular method confirmed the specific identity of the flies and proved to be highly specific, sensitive and permitted rapid identification of fly species.

The use of entomopathogenic nematodes (EPNs) *Heterorhabditis indica*, *Steinernema carpocapsae*, *S. glaseri*, *S. feltiae* and *S. abbasi* in biocontrol of housefly was undertaken. The survivability of EPNs in poultry manure at different time intervals revealed that, *S. feltiae* showed higher survivability in poultry manure followed by *H. indica*, *S. carpocapsae*, *S. glaseri* and *S. abbasi*. All the nematode species were able to cause 100% mortality in *Galleria mellonella* even after being exposed to the manure for 24 h. *G. mellonella* is a greater wax moth or honeycomb moth belonging to the family Pyralidae which was used for mass multiplication of EPNs in the laboratory. Eggs and pupae of all flies were found to be resistant to EPNs, whereas second and third instar larvae were susceptible to all the five EPNs. The LC_{50} and LC_{90} values indicated that *S. carpocapsae* was more virulent against second instars compared to other EPNs. *H. indica* was more virulent against third instars of *M. domestica* and also it was more virulent against second and third instars of *M. domestica* with artificial diet. In poultry manure, *H. indica* caused mortality of 25%, followed by *S. carpocapsae* 6.25%. Other three EPNs viz. *S. glaseri*, *S. abbasi* and *S. feltiae* did not cause any mortality. Among the five EPNs studied, the least progeny production in housefly larva was recorded for *S. abbasi* and high progeny production of *H. indica*. In the present study, it was found that EPN isolates were not potential biocontrol agents against house fly *M. domestica* in poultry manure probably because of presence of ammonia and other substances.

Biopesticides such as sweet basil, cumin seed, neem and curcumin were evaluated for housefly control. In fumigation assay, LC_{50} of cumin seed (0.018mg/cm³ adults and 0.08mg/cm³ larvae for 2h and 24h, respectively) were lower than the basil (0.030mg/cm³ adults and 0.10mg/cm³ larvae for 2h and 24h, respectively) suggesting cumin seed was

more effective than basil against house fly larvae and adults. The LC_{50} of basil (0.07 mg/cm^3) were lower than the cumin seed (0.11 mg/cm^3) suggesting basil was more effective than cumin seed against pupae. Basil (100 percent inhibition rate) was found more effective than cumin seed (73.33 PIR) against eggs. There was no fumigant toxicity of curcumin and neem on housefly egg, larva, pupa and adult.

In topical contact toxicity bioassay the LC_{50} of basil (0.046 mg/cm^3 for 2h) was lower than the cumin seed (0.059 mg/cm^3 for 2h) indicating that basil was more effective than cumin seed against adults. The LC_{50} of cumin seed (0.137 mg/cm^3 for 2h) was lower than basil (0.184 mg/cm^3 for 2h) suggesting cumin seed was more effective than basil against larvae. The LC_{50} of basil (0.25 mg/cm^3) was lower than the cumin seed (0.30 mg/cm^3) suggesting basil was more effective than cumin seed against pupae. There was no topical contact toxicity of curcumin and neem on housefly larva, pupa and adult.

In feed toxicity test cumin seed caused more mortality of house fly larvae than basil. There was no feed toxicity of curcumin and neem on house fly larva. In residual film toxicity assay basil was found to be more effective than cumin seed against larvae and adults of house fly. Neem caused 100% mortality in this method against larvae and adults of house fly at 1033 ppm/cm^3 whereas Curcumin did not cause any mortality.

The repellency of housefly larvae with basil and cumin seed at $25 \mu\text{l}$ of feed was found to be 100% repellent at 5min to 1hr. Neem was not repellent, whereas curcumin was 100% repellent at 30min and 1hr. All essential oils showed oviposition deterrent activity against housefly females, with cumin seed being highly effective. Among the four different essential oils used sweet basil and cumin seed were found to be very

effective in housefly control. As the sweet basil is cheaper and readily available than cumin seed, sweet basil can be considered as the best choice for the control of house fly.

The rate of dissipation of gel matrix upto 4hr was minimal where as the dissipation was more in 24hr and onwards at room temperature (35⁰ C and 40⁰ C) with complete loss of moisture after 48hr. In field assay upto 48hrs more flies were attracted and killed by bait containing Z-9-tricosene as attractant and imidacloprid as insecticide compared to other attractants and quick bite. Imidacloprid is a systemic insecticide which acts as an insect neurotoxin and belongs to a class of chemicals called the neonicotinoids which act on the central nervous system of insects, with much lower toxicity to mammals. The method of semiochemical use for fly control revealed that (Z)-9-Tricosene was found to be good attractant compared to other attractants indicating its effectiveness to lure house flies. Imidacloprid was found to be a powerful insecticide for housefly control.

Diatomaceous earth did not cause any mortality of house flies both in artificial diet and in poultry manure.

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VII. BIBLIOGRAPHY

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Abstract



VIII. ABSTRACT

A study was conducted to know the prevalence status of flies in poultry farms of Bangalore districts by both morphological and molecular methods of identification. A total of six different flies were found to be prevalent viz. *Musca domestica*, *Chrysomya megacephala*, *Hydrotaea aenescens*, *Hydrotaea capensis*, *Hermetia illucens* and *Sarcophaga ruficornis*. In the rainy season all six different flies were prevalent whereas in summer and winter only *M. domestica*, *C. megacephala* and *Hermetia illucens* were found. In deep litter system of rearing only *M. domestica* was prevalent whereas in cage rearing all six different flies were found. CO1 of six different flies was amplified at 658bp. Sequence of these species after BLAST analysis confirmed the species which was identified based on morphology. Sequence of these species submitted to GenBank and barcode was generated. The molecular method proved to be highly specific and sensitive and permitted rapid identification of flies. Entomopathogenic nematodes (EPNs) were evaluated in the biocontrol of house flies. After exposing to poultry manure, *Steinernema feltiae* showed more survival followed by *Heterorhabditis indica*, *S. carpocapsae*, *S. glaseri* and *S. abbasi* in all exposition periods (24, 48, 72 and 96h). When the exposition period extended to 96 h, the nematode survivability was drastically reduced. After exposing these nematodes to poultry manure at 24h, their virulence capacity against wax moth, *Galleria mellonella* showed that all the nematode species were able to cause 100% mortality. However their progeny production was significantly reduced. Fly eggs and pupae were refractory to the EPNs. Petri dish without artificial diet assay showed that, second and 3rd-instar larvae were highly susceptible to EPNs as compared to larvae provided with artificial diet. *H. indica* showed high virulence capacity compared to other nematodes tested. Poultry manure assay revealed that, *H. indica* and *S. carpocapsae* caused minimal mortality whereas *S. feltiae*, *S. glaseri* and *S. abbasi* did not cause any

mortality. This may be because of poor survival and limited movement of nematodes in poultry manure which may be due to ammonia and other substances in poultry manure. The decrease in larval mortality in manure suggested that biocontrol of housefly by using EPNs is unlikely. *H. indica* produced significantly more number of infective juveniles per insect larva than the other nematode species. Among the five EPNs studied, the least progeny production was recorded for *S. abbasi*. Different biopesticides were evaluated for housefly control viz. sweet basil, cumin seed, neem and curcumin. Based on the LC_{50} in fumigant toxicity assay, cumin seed (0.08 and 0.018 mg/cm³ for larva and adult, respectively for 2h) was found to be more effective against house fly larva and adult whereas basil (100 PIR-percent inhibition rate) was found to be more effective against egg and pupa. In topical contact toxicity assay cumin seed was found to be more effective on housefly larva whereas basil was more effective against adult and pupa. In feed toxicity cumin seed was found to be more effective. In fumigant, topical and feed toxicity assay neem and curcumin were not effective against any stage of house fly. In residual film coat, basil was found to be more effective compared to cumin seed whereas neem was effective only at highest dose on larva and adult. Curcumin was not effective against any stage of house fly. Basil and cumin seed showed 100% repellency of house fly larvae in 5min upto 1hr. Curcumin showed repellency at 30min to 1hr. Neem did not show any repellency. All essential oils showed oviposition deterrent activity against housefly females. Cumin seed showed highest percentage of effective repellency (ER%) followed by basil. Neem and curcumin did not show desirable repellent effect. Basil and cumin seed had very good insecticidal property against egg, larva, pupa and adult houseflies. In the semiochemical method of control it was observed that Z-9-tricosene incorporated bait with imidacloprid attracted and killed more flies compared to other attractants and quick bite. Evaluation of diatomaceous earth did not indicate any effect on house flies when fed in the diet or applied in poultry manure.