1. INTRODUCTION

The housefly, *Musca domestica* L., a wide spread filthy medical and veterinary pest, is closely related with the human settlement. High densities of houseflies lead to irritate farm workers, public health staff. Houseflies are responsible for sporadic burst of various pathogenic diseases, a stress pattern in livestock and fowls, leading to their physiological and behavioural changes (Kumar *et al*.,2012, Pavela, 2008). In cow shed, house flies reduced milk production of cows, farm worker efficiency and increased frequency of animal disease transmission, leading to enhanced medication veterinary service costs (Albarrk,2009).Overall its potential to spread of human diseases increases (Douglass and Jesse 2002). More than 100 pathogens associated with the house fly may cause diseases in humans and animals (Ojianwuna *et al*., 2011). These diseases include typhoid, cholera, tuberculosis anthrax, mastitis, bacillary dysentery and infantile diarrhoea (Service, 1980).

Conventional methods for insects-pests control depend on the use of insecticides (Sukontason *et al*., 2004). Although chemical insecticides can effectively reduce fly populations (Albarrak, 2009) but some serious side effects from these chemicals due to the residuals found in food, the environment, and non-target organism have been noted. Long half- life of insecticides in the environment could create an accumulation of chemicals, which are found in food chains or food webs finally affecting the humans and animals. According to World Health Organization (WHO) estimation annually 2,20,000 deaths occur due to acute poisoning caused by synthetic pesticides. Insecticides and pesticides will not only reach the target organisms but also kill other organisms (e.g. beneficial insects, birds, earthworms, fish) in or around the crop fields, causing loss of biodiversity, deaths of wild life, and death of farm animals. Soil, air and water bodies can easily be contaminated with these poisonous

chemicals. Insecticide also kills the natural enemies of these insects. That means that natural control mechanisms are disrupted and it allows the insect population to rapidly build up again to levels that can cause serious problem like pest resistance and pest resurgence. The high cost of chemical pesticides and the environmental hazards as a result of pesticide usage have encouraged scientists to search for less hazardous and cheaper pesticide groups (Siriwattanarungsee *et al*., 2008).

The demand for chemical pesticides can be reduced by use of bio-pesticides that will in term reduce the load of synthetic chemicals in the environment. The use of plant products in the control of insect species depends on bioactive substances that inhibit the developmental process of those insects (Kristensen and Jespersen, 2003). Botanical insecticides based on natural compounds from plants, are expected to be a possible alternative. They are relatively specific in their mode of action and easy to process and use (Bisseleua *et al*., 2008).

In the search for safer pesticides, research on the potential of Neem products has been conducted internationally (Khan and Ahmed, 2000a, 2000b). The use of Neem (*Azadirachta indica* A. Juss) as bio-pesticides is safer. Neem and its products have no ill effects on humans and animals, and there is no residual effect on agricultural produce. This makes Neem as one of the best alternative to hazardous pesticides. Literature survey reveals that the oil extracted from seeds of *Azadirachta Indica* is found to have biocidal activity against nearly 200 medical and veterinary arthropods, without any adverse effects toward most non target organisms (Naqvi *et al*., 2007). However studies on the use of Neem oil for housefly control are very limited. In view of the above facts, objective of the present study was to do a comparative analysis of the essential Neem oil for the control of house fly in the laboratory condition with the optimization of dosage, followed by testing the results in semi controlled condition (Azima *et al*. 2011).

2. LITERATURE REVIEW

The housefly is cosmopolitan, it makes up 98 percent of flies that invade homes, and is considered one of the filthiest insect pests (Ojianwuna *et al*., 2011). Housefly belongs to the insect order Diptera is related to mosquitoes and gnats. Diptera means two ("di") wings ("ptera"), and it is on the basis of this single characteristic (one pair of wings) that all the species of flies are grouped together. Flies are cold-blooded insects that move about looking for external heat sources; most flies are diurnal and are attracted to certain wavelengths of light.

2.1. Housefly Life cycle

The housefly is a non-biting fly measuring about 1/4 inch long. Adult houseflies have two wings and four lengthwise black stripes on their backs. The abdomen typically appears checkered. Eggs are usually laid in masses on organic material such as manure and garbage. Hatching occurs within a few hours. The young larvae burrow into the breeding material; they must obtain oxygen from the atmosphere and can, therefore, survive only where sufficient fresh air is available. When the breeding medium is very wet they can live on its surface only, whereas in drier materials they may penetrate to a depth of several centimetres. They hatch after about 12 hours and the larvae, or maggots, feed on the rotting organic material. A maggot passes through three larval stages and then forms a pupa, or cocoon. The adult fly emerges from the cocoon. Houseflies typically develop from egg to adult within 10 days (Fig 2.1). Depending on the temperature, it takes from 6 to 42 days for the egg to develop into the adult fly. The length of life is usually 6–10 weeks (Axtell and Arends 1990) but under unfavourable conditions it may be as long as three months (Sacca 1964).

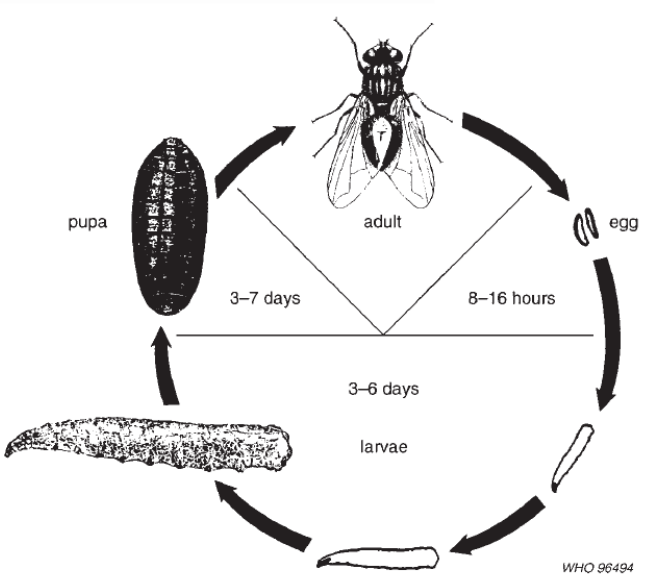

Fig. 2.1. Life cycle of House fly

2.1.1. Egg

The eggs of housefly are white, elliptical, about 1mm long by 0.26 mm wide, with both ends bluntly rounded and the anterior slight. The maximum egg production occurs at intermediate temperatures, 25 to 30°C. Eggs are deposited by the mated female 4–8 days after copulation, while hatch within 24 hours of deposition. An unmated, virgin female may lay a few eggs, but these will not hatch. The female selects a suitable oviposition site and deposits her eggs in one to several masses. The number of eggs which will mature in the ovaries at one time is 100–150 (average=120). A female typically deposits 4–6 batches of eggs in her lifetime.

2.1.2. Larva

Larvae and pupae are most dominant life stages of house flies, in terms of population percentage and also reflect secondary impact of essential oils to control insect population (Kumar et al., 2012).The house fly larva consists of an outer cellular cuticle and an inner single layer of epithelium which rests on a basement membrane. The cuticle is covered with

an epicuticle and has a stratified structure. The cuticle is 5µm thick in young larvae (36 hours old), 25µm in 60-hour-old larvae and 40µm in last 3rd instar larvae.

The larva is white and cylindrical, with the posterior end broad and flattened. It tapers anteriorly. There are no eyes or appendages, although there are some ventral spiny ridges which aid locomotion. The larvae have 13 segments, but the first two are partially fused so that only 12 segments are apparent. The spiracles are openings for air to enter the respiratory system of the larva, with distinctive posterior spiracles.

2.1.3. Pupa

During pupation, the larva contracts within its own integument so that the integument becomes a cylindrical puparium about 6.3 mm long. The puparium gradually darkens to a rich, dark brown color. Since the pupal case is formed by the larval skin, the pupa within is said to be coarctate. The pseudocephalon is completely withdrawn, resulting in the anterior spiracular processes being very near the anterior end of the puparium. Locomotor pads persist on the ventral surface although the puparium is immobile.

2.1.4. Adults

Adult houseflies have been shown to transmit pathogens from their sponging mouthparts, through vomitus, on their body and leg hairs, on the sticky parts of the feet, and through the intestinal tract, (De Jesus et al., 2004) thereby contaminating food and propagating disease (Palacios et al., 2009). The house fly is 6 to 7 mm long, with the female usually larger than the male. The female can be distinguished from the male by the relatively wide space between the eyes (in males, the eyes almost touch). The head of the adult fly has reddish-eyes and sponging mouthparts. The thorax bears four narrow black stripes and there is a sharp upward bend in the fourth longitudinal wing vein. The abdomen is gray or yellowish with

dark midline and irregular dark markings on the sides. The underside of the male is yellowish.

Adults usually live 15 to 25 days, but may live up to two months. Without food, they survive only about two to three days. Longevity is enhanced by availability of suitable food, especially sugar. Access to animal manure does not lengthen adult life and they live longer at cooler temperatures. They require food before they will copulate, and copulation is completed in as few as two minutes or as long as 15 minutes. Oviposition commences four to 20 days after copulation. Female flies need access to suitable food (protein) to allow them to produce eggs, and manure alone is not adequate. The potential reproductive capacity of flies is tremendous, but fortunately can never be realized.

2.2. Control of housefly

Control is a combination of reducing exposure to pest and treatment with effective products to lessen the effects. The control of pests, insects and disease, either directly or indirectly, using natural plant products or botanicals, including essential oils, is promising (Regnault-Roger, 1997; Isman, 2006). Housefly management relies heavily on sanitation facility, screening measures and pesticide application. However, these are often difficult to implement because of high labour costs and the impracticability of screening methods (Kumar *et al*., 2011).

2.2.1. Sanitation or cultural control

Good sanitation is the basic step in any fly management program. The control of *M. domestica* is thus, vital to human health and comfort (Tilak *et al*., 2010). Food and materials on which the flies can lay eggs must be removed, destroyed as a breeding medium, or isolated from the egg-laying adult. Since the house fly can complete its life cycle in as little as seven

days, removal of wet manure at least twice a week is necessary to break the breeding cycle. Wet straw should not be allowed to pile up in or near buildings. Since straw is one of the best fly breeding materials, it is not recommended as bedding. Spilled feed should not be allowed to accumulate but should be cleaned up two times a week. Ordinarily, fly control from 1 to 2 km around a municipality prevents house fly infestations.

2.2.2. Physical control

Physical control of houseflies includes natural fly traps (Ojianwuna *et al*., 2011). Fly traps may be useful in some fly control programs if enough traps are used and are placed correctly (Ojianwuna *et al*., 2011). House flies are attracted to white surfaces and to bait that give off odors. Indoors, ultraviolet light traps collect the flies inside an inverted cone or kill them with an electrocuting grid. One trap should be placed for every 30 feet of wall inside buildings, but not placed over or within five feet of food preparation areas. Recommended placement areas outdoors include near building entrances, in alleyways, beneath trees, and around animal sleeping areas and manure piles. Openings to buildings should be tightly screened with standard window screen, thereby denying entrance to flies. Traps can be baited with molasses, sugar, fruit or meat, and often are used in combination with a device that captures the attracted flies. The sex pheromone (Z)-9-tricosene also functions as an aggregation pheromone, and is called muscalure. Muscalure is formulated with sugar as a commercially-available fly bait for local population suppression, as well as an enhancement for population monitoring.

2.2.3. Chemical control

When the house fly is a major pest in commercial egg production facilities, the control of this insect is by the application of adulticides, or larvicides to directly or indirectly suppress

adult densities. Residual wall sprays can be applied where the flies congregate. Outdoors, the control of flies includes the use of boric acid in the bottom of dumpsters, treatment of vertical walls adjacent to dumpsters and other breeding sites with microencapsulated or wettable powder formulation, and the use of fly baits near adult feeding sources.

However, use of chemical insecticide is not only detrimental to environment but its long time use also leads to development of resistant among insects (Srinivasan *et al*. 2008; Kamaraj *et al*. 2011). Pyrethroids were initially highly effective on the housefly control (Scott, and Kasai, 2004). Unfortunately, very high levels of resistance to this class of insecticides have developed worldwide due to intensive use over the last 30 years (Hoton *et al*., 1985; Chapman *et al*., 1993; Pap and Farkas, 1994; Scott *et al*., 2000). Resistance to permethrin develops more rapidly in fly populations from farms on a continuous permethrin regime than in farms in which permethrin and diclorvos have been alternated. Also, considering the bio-habitat of house flies, insecticides favourable or at least compatible to human surroundings are needed which is not possible with chemical insecticides (Kumar *et al*., 2012).

2.2.4. Biological Control

With the increasing incidence of insecticide resistant house fly populations, rising costs of insecticides and a growing public concern about actual or potential problems associated with insecticides, interest in alternative house fly control strategies has increased. The use of safer alternatives like biological control or insect growth regulators (IGR) is thus gaining attention as an important intervention in housefly management programmes (Axtell and Arends 1990; Tunaz and Uygun 2004). The use of biological control agents in fly management programs is still at a relatively early stage. House fly has many natural enemies and among the most important in poultry facilities are the wasps (Hymenoptera: Pteromalidae) *Muscidiforax*

raptor and *Spalangia cameroni* (Ojianwuna *et al*., 2011). At present, parasitic wasps are the most widely used biological control agents for house flies.

2.2.4.1.Plant essential oils in biological control

Plant essential oils and their components have been known to exhibit biological activities, especially antimicrobial, since ancient time (Kumar *et al*., 2011). The ancient Egyptians, Greeks and Romans knew peppermint as flavouring agent for food and as medicine while the essential oils of mint were used as perfumes, food flavours, deodorants and pharmaceuticals (Barıs *et al*., 2006). In recent years, essential oils and their components are gaining increasing interest due to being relatively safe for the environment as well as to the human health, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey *et al*., 2001). Various studies have reported the use of essential oil against various stages of household pets; such as housefly and mosquitoes. The detailed reports on the efficacy of different essential oils against housefly and mosquitoes are summarized in Table 1.

Among essential oils, Neem (*Azadirachta indica*) is a tropical evergreen tree native to India and is also found in other southeast countries. In India, Neem is known as "the village pharmacy" because of its healing versatility, and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. The seeds, bark and leaves (Fig. 2.2) contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal uses. The earliest documentation of Neem mentioned the fruit, seeds, oil, leaves, roots and bark for their advantageous medicinal properties.

Figure 2.2. The Neem Products. (**A**) Twigs, (**B**) Leaves, (**C**) Fruits, (**D**) Seeds (with endocarp), (**E**) Seeds (without endocarp).

Recent experiments have shown that one of the Neem's components, gedunin (a limonoid), is as effective as quinine against malaria. Neem oil treated mosquito nets and mosquito-repellent cheap tablets (one paise per Tablet) are also becoming popular. Table 2 summarizes various reports on use of neem oil for controlling different insect-pest species.

3 MATERIALS AND METHODS

2.3.Housefly

Adult houseflies were collected from the garbage site of the Indian Institute of Technology, Delhi, India, using a sweep net method. These houseflies were reared in cylindrical boxes (90 × 140 mm) covered with muslin cloths in growth chamber maintained at 28 ± 2^{o}C and 65% relative humidity (RH). Rearing of house flies were done as per the method described by Kumar et al., 2011.

2.4.Rearing of house fly

Houseflies were reared in cylindrical boxes (90 × 140 mm) covered with muslin cloths and maintained at 28 ± 2 ◦C, 65% relative humidity (RH) in a growth chamber. During rearing, flies were fed on a mixture of groundnut oil cake and wheat bran at a ratio of 1: 3. Eggs were transferred to another box containing the same diet. Hatched larvae were transferred individually to cylindrical vials (28 × 12 mm) containing a semi-synthetic diet (constituents: 2 g groundnut oil cake, 5 g wheat bran, 2 g milk powder, 1 g honey mixed with 10 mL of water); this diet was changed daily until larvae reached the pupal stage to avoid any contamination. Pupae were transferred to Petri plates containing no diet or moisture. Field-collected flies were used in the repellency bioassays; larvae and pupae obtained from the rearing of these field-collected flies were used in the larvicidal and pupicidal bioassays

2.5.Source of essential oil

Essential oil of Neem (*A. indica*) used in the study was procured from the local market, New Delhi, India and was stored in plastic bottles at 4^{o}C.

2.6. Bioassay against housefly

2.6.1. Repellency assay

The repellent effects of Neem oil was evaluated against field-collected adult houseflies (n=30-40) from the garbage site of the Indian Institute of Technology, Delhi. Chamber consisting of an outer size (20×20×20 cm) were used for repellency assays. Petri plates (90 mm) containing filter paper impregnated with Neem oil was used for the repellency assay in the chamber. This chamber was connected to an inner chamber (60×60×60 cm) by a hole measuring 9×9 cm (Fig. 3.1).

Figure 3.1. Repellency Chamber

(Kumar et al., 2011)

Flies that moved to the inner chamber were counted as having been repelled. The 5 different concentrations of Neem oil (100, 200, 300, 400 and 500 µl) diluted with 500 µl of acetone were used for bioassay. The experiment was performed for 2 h, at the end of which the number of flies repelled was counted to calculate the percentage repellency. A control treatment using 500µl acetone alone was also conducted. Treated and control discs were air-dried for 5 min to allow the acetone to evaporate completely.

2.6.2. Larvicidal bioassays

Larvicidal bioassay was performed by contact toxicity assay. Housefly larvae were obtained by rearing the field-collected flies. Larvicidal bioassay was performed against both; 2nd Instar and 3rd instar housefly larvae in two separate set of experiments. For each larvicidal bioassay, 10 larvae (second instars) were placed on a filter paper (in each Petri plate) containing a diet of 2 g groundnut oil cake, 5 g wheat bran, 2 g milk powder and 1 g honey mixed with 10 ml water (Kumar *et al*. 2011). Different volumes of Neem oil was mixed with 500 µl acetone to correspond to the Neem oil concentrations of 0.16, 0.31, 0.47, 0.63, and 0.79 µl/cm^2, and were applied to the diet in a pour-on treatment (Fig. 3.2). Treated filter paper was air dried for 5 min before putting on larvae. Each oil treatment was replicated thrice. Control filter paper was sprayed with acetone. All bioassays were performed at 30±2°C and RH 65± 5%. Larval mortality was assessed by withering and the development of a brownish appearance (Kumar *et al*. 2011).

Figure 3.2. Larvicidal assay

2.6.3. Pupicidal bioassays

Pupicidal bioassay was performed by contact toxicity assay. For pupae bioassay, 10 pupae without any diet supplement were placed on a filter paper (in each Petri plate).

Different volumes of Neem oil was mixed with 500 µl acetone correspond to Neem oil concentrations of 0.16, 0.31, 0.47, 0.63, and 0.79 µl/cm^2, and were applied to the diet in a pour-on treatment (Fig. 3.3). Treated filter paper was air dried for 5 min before placing on larvae. Each oil treatment was replicated in thrice. The petri plate only sprayed with 500 µl acetone was kept as control. Before placing to larvae, the plate was kept it for five minutes for drying. All bioassays were performed at 30±2°C and RH 65± 5%for 24 hours.

Figure 3.3. Housefly pupae treated with Neem oil

The rate of pupicidal activity of the oil was calculated as the percentage reduction in adult emergence or inhibition rate (Kumar et al., 2011b). Inhibition rate (% IR) was calculated as:

$$\textbf{Inhibition rate (\% IR)} = \frac{Cn - Tn}{Cn} x100$$

Where Cn is the number of newly emerged insects in the untreated (control) Petri plates and Tn is the number of insects in the treated Petri plates.

2.7. Encapsulated bead formulation using Neem oil

2.7.1. Preparation

The encapsulation of Neem oil at 5% conc. was performed using 1.5 g (3%) of sodium alginate in 50 ml of distilled water. The mixture was heated to 70 ^{0}C to melt Na-alginate and

after melting the mixture was kept for cooling. 2.5ml Neem oil was added to this mixture before solidification with continuous mixing on magnetic stirrer.

Further, 50 ml of the 2% $CaCl_2$ solution was taken in a beaker and 0.5 ml of the formaldehyde was added to this and mixed completely. The mixture of the Na alginate and Neem oil was taken in a 10 ml syringe for beads preparation. The alginate mixture was poured drop wise in the $CaCl_2$ solution prepared. The beads were filtered using filter paper and kept in the petri plates for drying it in B.O.D. incubator at 28 ^{0}C for 48 h. In the similar way, 10% of the Neem oil was trapped in the Na alginate beads (Fig. 3.4).

Figure 3.4. Sodium alginate beads of Neem oil

2.7.2. Release study of Neem oil beads

For the release study of Neem oil beads, 1gm of 5% and 10% Neem oil beads prepared in two different beakers containing 50ml methanol in each and shake it for half an hour on shaker. 50ml of methanol as control was also run parallel. The release of the oils from 5% and 10% Neem oil entrapped beads was monitored using spectrophotometer at 254 nm after regular interval of half an hour for four hours. The data for 5% and 10% Neem oil was

recorded & compiled with control curve. The concentration of oil released from the beads was determined using the standard calibration curve prepared using 10-100µl of the Neem oil in the methanol.

2.7.3. Effect of Neem beads against housefly 3rd instar larvae

The effect of the Neem oil beads (5% & 10%) was evaluated against housefly larvae (3rd instars) in a petri plates bioassay. Larvae (10 in no.) were placed in a Petri plate containing diet materials (mentioned in the section 3.4.2) along with Neem beads (1gm). Each treatment was replicated thrice. For control experiment, alginate beads containing no oil were used. All bioassays were performed at 30±2°C and RH 65± 5%. Larval mortality was assessed by withering and the development of a brownish appearance (Kumar *et al*. 2011).

3. 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_{90} and LT_{90} (Finney 1971; SPSS 2008) and Cluster analysis of the based on distance matrix was determined by (PAST) software.

4. RESULTS AND DISCUSSION

4.1. Repellency assay

The insecticidal effects of Neem oil against *M. domestica* adults were evaluated by determining the repellency (%), which is presented in Fig. 4.1. The mortality rate percentages of adult housefly were highly significant at different doses (100 µl to 500µl) of Neem oil. The maximum repellency and mortality was recorded 82.33% and 50 %, respectively at 2h in repellency test supplemented with 500 µl of Neem oil. The figure clearly indicated that more than 80% of the repellency can be obtained at 500 µl Neem oil concentration after 2 hrs of the incubation.

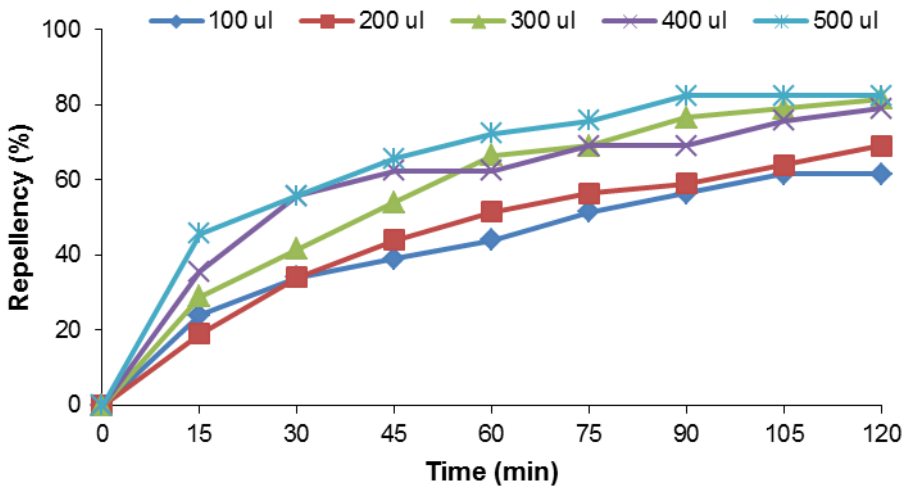

Figure 4.1 Repellency (%) of adult housefly with different concentrations of Neem oil

Comparing the RD_{50} for most of the applied dose it was found that the time required was reduced to less than 30 minute at 500µl of the Neem oil. The figure clearly indicated that RD_{50} value was obtained after 90 minutes of the observation period at 100µl of the Neem oil. However, in control run 37.5 % repellency and 7.5 % mortality was recorded. The earlier

literature also reported that more than 85% of the mortality of the adult house fly after exposure to Neem extract can be obtained. (Khan and Ahmed 2000).

Although larger lethal dose requirement of essential oils has been implicated, other important effects at sub-lethal doses may play significant role in overall insect bio-control (Pavela, 2007). Sub-lethal doses of essential oils have been indicated for growth inhibition, weight loss and high agitation in insects' larvae (Hummel, and Isman, 2001). Even if larger dose of essential oils may be required for insects' lethality, consequence of sub-lethal dose on their behaviour, although not very prominent, might manifest overall biocontrol (Kumar *et al*, 2012). Previous studies have also supported high efficacy of neem oil against insect-pest. The complete protective nature (i.e. no confirmed bites) of the neem oil, when formulated as 2% in coconut oil, against *Anopheles* mosquitoes, for 12 hours was achieved (Sharma *et al*., 1993).

The data analysed by principal component analysis (Fig.4.2) showed the effect of the Neem oil concentration with time. The separately and opposite placed control data indicating, that there is no effect on the control with passage of the time. The repellency rate at the concentration 400 µl and 500 µl are grouped together in the vicinity of the 45 minute incubation period. Similar observations were noted for the oil concentration of 200 µl and 300 µl, indicating similar effect on the repellency of the adult fly. The principal component analysis not only helps in the clustering of the similar data but also assists in the identification of the effectiveness of the particular variable.

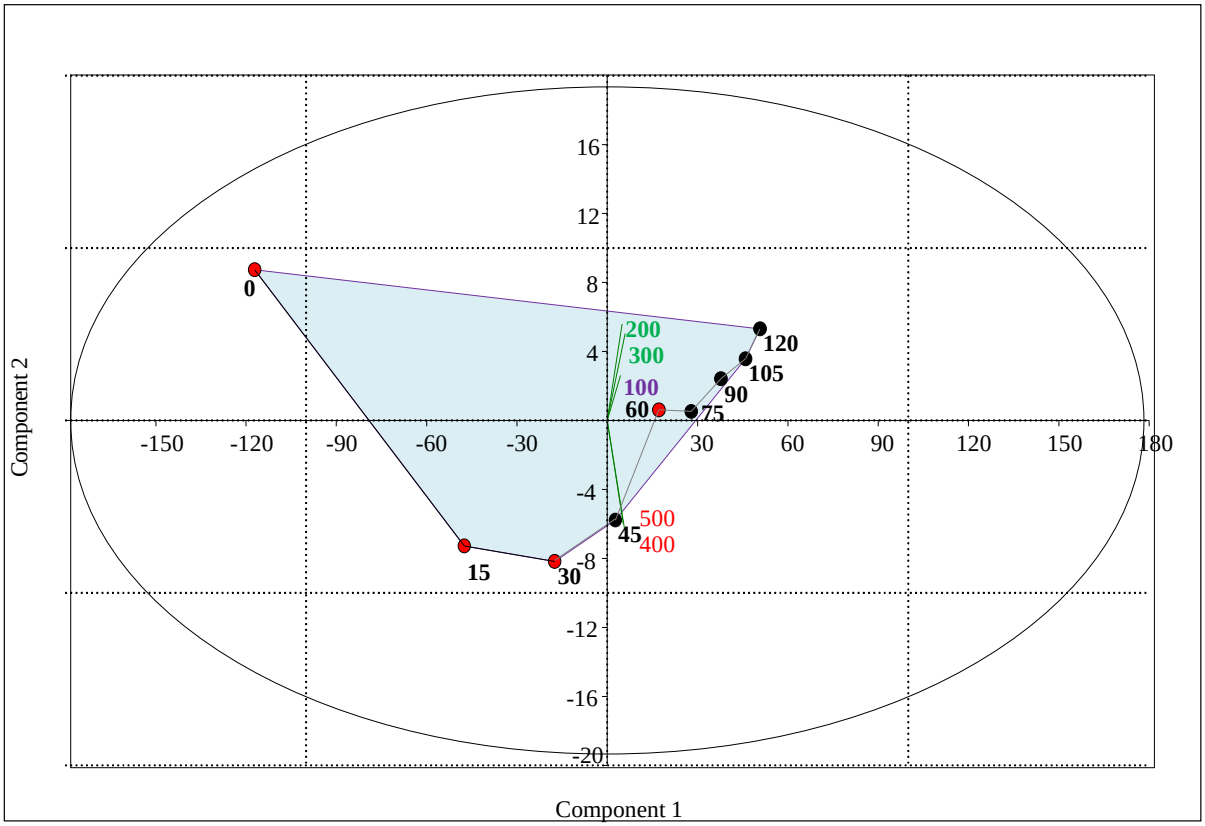

Figure: 4.2 Represents the distribution of the clustering of the dose effect on the repellency versus time.

Principal component analysis also results in various clusters in the ensuing graph. In the present graph 2-3 clusters are apparent. The graph also gives an idea about the situation of any individual treated subject (in this case, adult housefly) during the treatment period. Points that are near in the graph represent similar situation of flies during treatment procedure. This point prediction helps in comparing the effect of one house fly with the other flies. Examination of the scores and loadings plots for PC1 (Neem oil concentration) vs. PC2 (repellency versus time) (Fig.4.2) showed good experimental replication since tight clustering of replicate samples could be seen with several of them clustering on top of each other. PC1 accounted for 98.3% of the variance. The positive value of the covariance (98.3) indicated

that both dimensions increase together, meaning that, in general, as the concentration of the Neem oil increased, the rate of the repellency increased.

Examination of the scores plot demonstrated that control was separate from rest of the group. The separation for the control in the figure is evidenced mainly by virtue of its growth in the absence of Neem oil relative to the other group. Examination of the scores plot also indicated that the rest of the set of fly had fairly similar levels and further related in the two groups. Thus it would appear that higher repellency could be achieved at elevated concentration levels of Neem oil. These patterns showed that deviations from the control range increase over time for both exposure concentrations and are similar in the direction of response.

4.2.Bioassay on 2nd instar and 3nd instar larvae with Neem oil

Larvicidal activity of Neem essential oil through contact toxicity assay is presented in Fig 4.3 and Fig. 4.4. The highest percentage mortality of 2nd and 3rd instar larvae was similar (30%) at 0.63µl/cm^2 and 0.79µl/cm^2 dose in the 5% neem oil concentration. The suppression rate of adult emergence observed was 53.3 % for 2nd and 3rd instar larvae at 0.63µl/cm^2 and 0.79µl/cm^2 Neem oil concentration after 24h of exposure. The LC_{50} and LC_{90} of both 2nd and 3rd instar larvae observed are mentioned in Table 3. After 6 days of observation the larvae turned to pupae that subsequently gave rise to normal adults. The treatment also revealed the morphological damages of considerable nature in treated larvae. The morphological damages were more apparent at the higher Neem oil concentrations. No larval mortality was recorded in the control treatment.

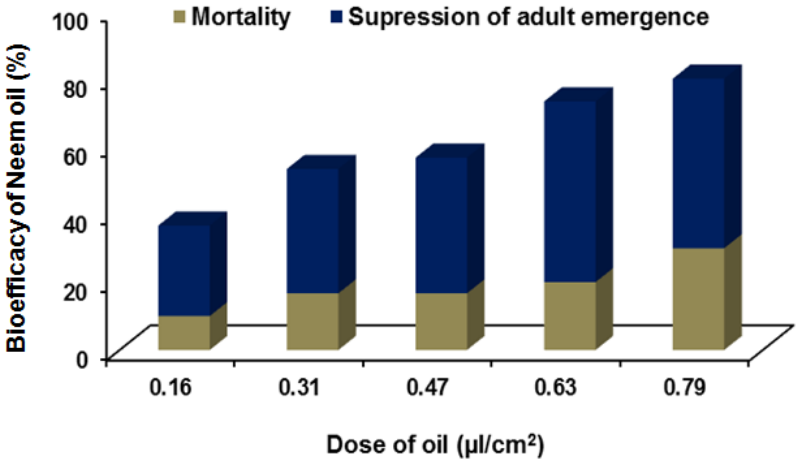

Figure 4.3 Effect of the Neem oil concentration on the repellency (2nd stage instar larvae)

The morphological damage during the treatment of the larvae with oil concentration was in agreement with the previous reports observed could be discerned by the observation of anterior region and spinose ring between segments using essential oils (Kumar *et al*., 2012) and surface structural changes in housefly larvae by application of eucalyptol through SEM image analysis and observed bleb formation, deformation in the integument and intersegmental spines of treated larvae (Sukontason *et al*. 2004).

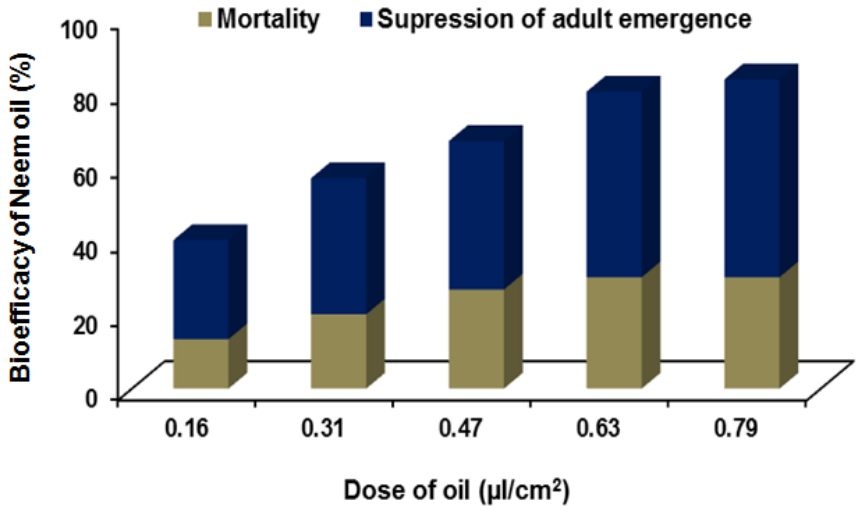

Figure 4.4 Effect of the Neem oil concentration on the repellency (3rd stage instar larvae)

Comparing the mortality and the suppersion of the 2nd instar and 3rd instar (Table 3) larvae it could be seen that there was no much significance differences in the mortality and the supresion rate.

Table 3. Lethal concentrations and Chi-squared value for mortality and suppression data through Neem oil treatment of 2nd and 3rd instar housefly larvae

2nd instar larvae	LC50	LC90	Chi-squared value
Mortality	1.35 (1.03-2.45)	2.59 (1.83-2.56)	1.411
Suppression	0.69 (0.57-0.93)	1.92 (1.44-3.27)	2.53
3rd instar larvae			
Mortality	1.27 (0.96-2.45)	2.67(1.85-6.12)	1.712
Suppression	0.68 (0.57-0.89)	1.84 (1.41-3.01)	0.727

Principal Component analysis was done for the larval mortality and suppression of adult flies emerged from the pupae obtained from treated larvae (both 2nd instar and 3rd instar) (Fig. 4.5). The scores plot of Principal Component 1 (94.66) vs. Principal Component 2 (4.35) showed the correlation between PCs 1 and 2 for the analysed samples after they have been differentiated according to surveys and locations. PC1, PC2, PC3 and PC4 explain 94.66, 4.35, 0.97 and 0.004 % respectively indicating that PC1 was the major component explaining the variance. This was also indicated by the higher eigen value i.e. 315.77. The amount of variance (i.e. information) spanned by each PC depends on the relative value of eigen value with respect to the total sum of eigen values (Helena et al., 2000).

A polygon view of the biplot drawn on mortality and suppression shows that all other larvae are inside the polygon while 2nd instar larvae are outside the polygonal. This larvae stage is the most responsive since they have the longest distance from the biplot origin. Responsive are considered as those that are either the best or the poorest in one or all environments (Yan and Rajcan, 2002). The 2nd instar larvae showed lesser mortality indicating the best adaptability to the environment.

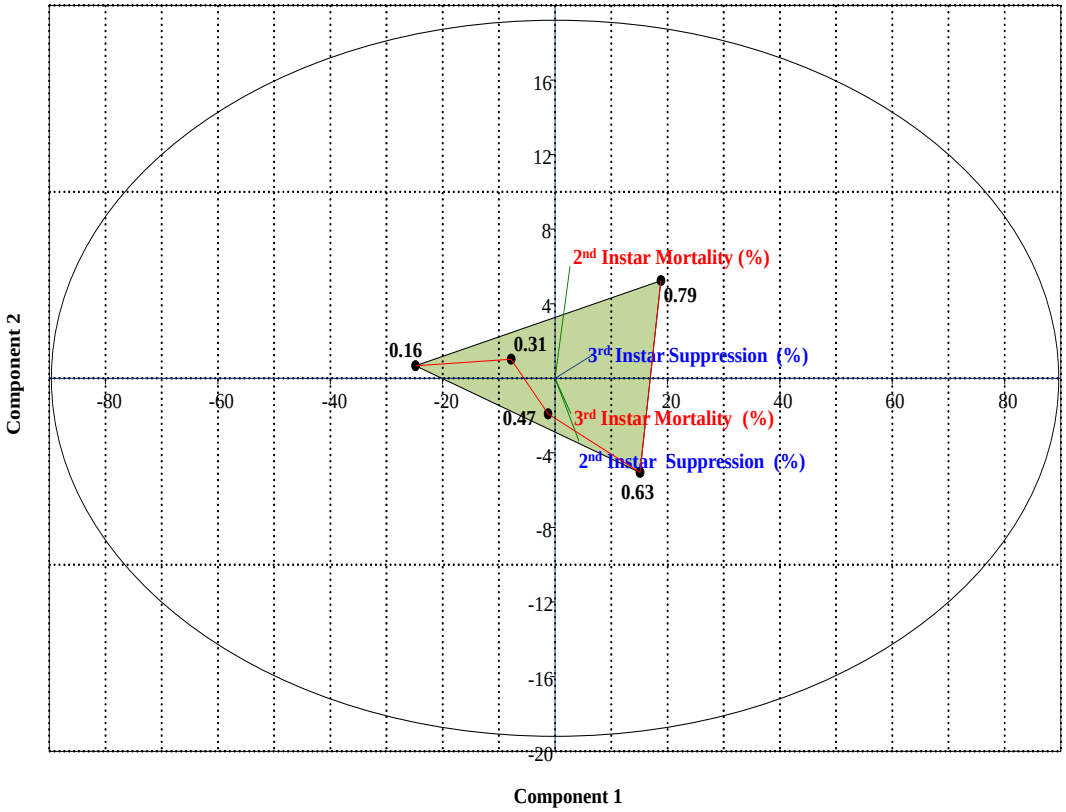

Figure: 4.5. The bioplot of the principal component analysis of the mortality and suppression of the larvicidal at 2nd and 3rd instar.

The mortality and suppression data was subjected to combined analysis of variance in the house fly population. Cluster analysis of the (Fig. 4.6) based on distance matrix was determined by PAST software. As the effect of the biopesticide dosing was significant hence the data was subjected to biplot. The figure indicates that the combine cluster analysis of mortality and suppression of the 2nd and 3rd instar larvae are grouped in two main clusters based on the dose level (µl/cm^2). The first cluster was again subdivided into three sub-clusters.

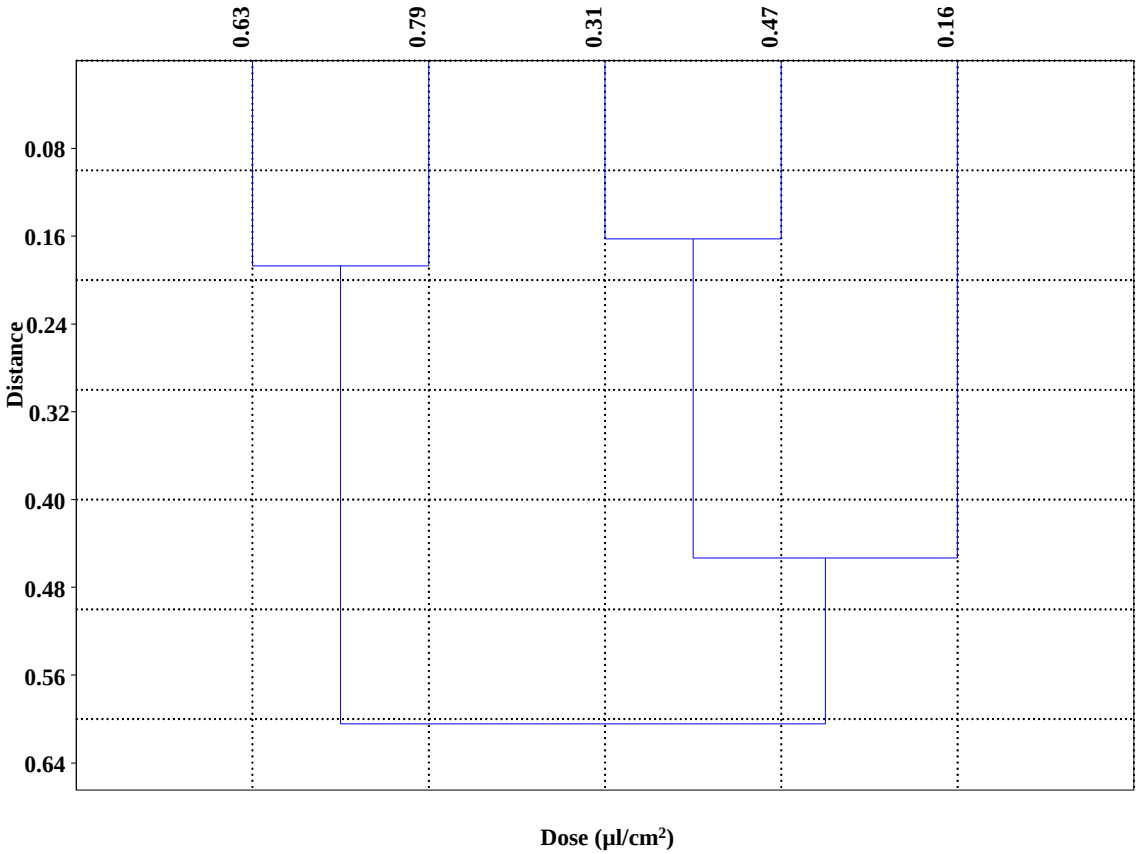

Figure: 4.6. Dendrogram resulting from a cluster analysis of the mortality and suppression of the 2nd and 3rd stage instar larvae.

4.3. Bioassay of housefly pupae with Neem oil

Pupicidal bioassay indicated that the Neem oil was majorly non-effective against housefly pupae with a mere 10-20% inhibition in adult emergence at the oil concentration of 0.16-0.79 µl/cm^2.

4.4. Release profile of Neem oil from beads

Standard curve showing the absorbance of Neem oil at increasing concentrations is represented in Fig. 4.5. The graph showed increasing absorbance with increase in oil concentrations.

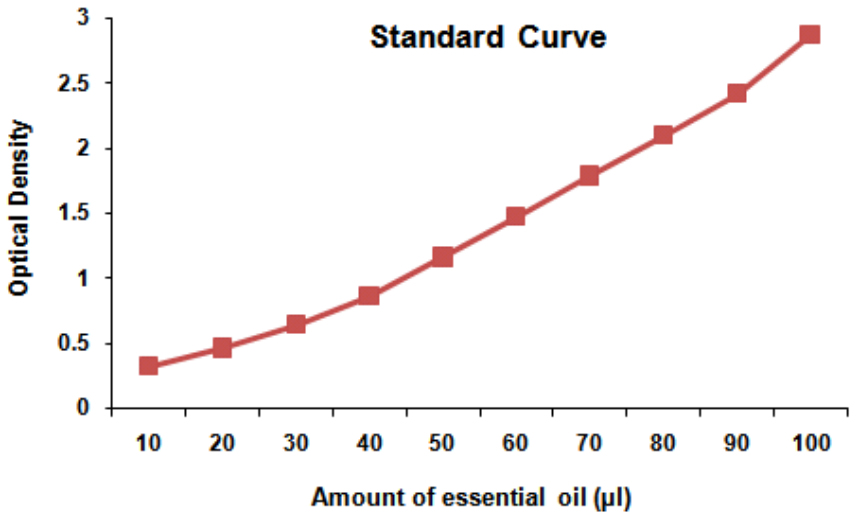

Figure: 4.5. The standard curve of Neem oil at 254 nm

The release of Neem oil from beads was slow. The rate of release of Neem oil 10 % during initial time is very less but at the end of 4h observation period, release of Neem oil increased abruptly.

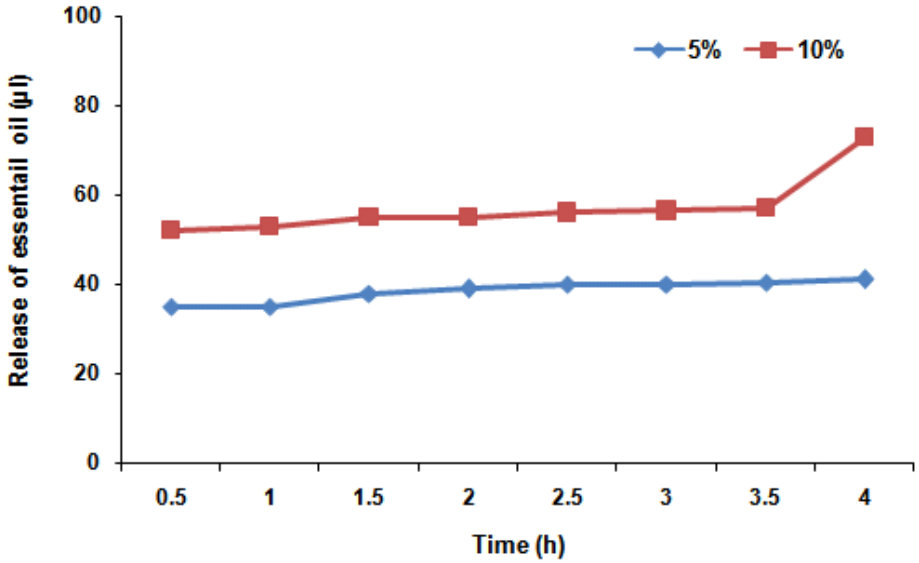

Figure 4.8. Release curve for Neem oil beads

4.5. Bioassay on housefly 3rd instar larvae with Neem oil beads:-

The percentage mortality of housefly larvae due to Neem beads (5% and 10%) is noted. The maximum mortality after 48 h of observation was 60% (10 % Neem oil beads) and 53% (5 % Neem oil beads). In the control, 6.6% mortality was observed after 48 hrs.

5. CONCLUSIONS

The successful control of the common house fly requires an integrated pest management approach. Larvae and pupae are most dominant life stages of house flies, in terms of population percentage indicating that secondary impact of essential oils to control insect population should be studied. The present study showed that Neem oil is good repellent agent against adult housefly. Neem oil treatment also showed fairly good efficacy against housefly larvae (2^{nd} and 3^{rd} instar), although the same control efficacy of Neem oil could not be observed for housefly pupae. The Neem oil encapsulated beads are stable under ambient condition for long exposure time with good effectiveness. It can be concluded that the plant based herbal ectoparasiticidal product is quite efficacious as a housefly repellent.

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