1. INTRODUCTION

There are 4600 species of cockroaches out of which 30 species of cockroaches are associated with human habitat. There are four known species of cockroaches that are considered as pests. They are German cockroaches, American cockroaches, Australian cockroaches and Oriental cockroaches.The American cockroach (*Periplaneta americana*) is the largest species of common cockroach which is native to the Africa and the Middle East. They were not endemic to America. But were introduced to the United States from Africa as early as 1625.

These cockroaches are omnivorous and are opportunistic feeders which feed on materials such as glucose, starch, paper, dead plants and animals and are also found to exhibit cannibalism. They generally live in cracks and crevices of buildings and basements, under doors, air ducts and drainage and are sensitive to light but prefer warmer environments with a temperature of 29°C.

Periplaneta americana has an average length of 4 cm and a height of about 7 cm with a life cycle of about one year and they moult 6 to14 times. They are reddish brown in colour and the margin of the pronotum is yellow in colour. It has a flattened body which is broadly oval and consists of three body segments namely the head, thorax and the abdomen. The head consists of a pair of compound eye made up of 2000 individual lenses which are hexagonal in shape called as the ommatidia. Below the pairs of eyes is present a pair of long, segmented antennae. The head segment also comprises of the mandible, a pair of labial and maxillary palp. The thorax is subdivided into prothorax, mesothorax, metathorax and a pair of walking appendages arises from each segment. The fore wings called as tegmina are leathery arising from the mesothorax which is dark and opaque and the hind wings are delicate arising from the metathorax and is responsible for flight. The head and a part of the dorsal surface of the thorax are covered by a shield-like pronotum. The abdomen is divided into 10 segments and is surrounded by a chitinous exoskeleton plates namely sclerites, dorsal tergites, ventral sternites and lateral pleurites (Plate 1).

Cockroaches are considered as household pests. It has the ability to produce odorous secretions which can alter the taste of food. They also carry bacteria such as Salmonella and contaminate food. House dust containing the faeces or body parts of this insect can cause asthma or trigger allergic reactions.

The genome of *Periplaneta americana* is the second largest genome to be coded among insects. It consists of genes for detoxification, immune system, growth and reproduction. Also, it contains 522 taste receptors out of which 329 are associated with bitter taste perception. It is the largest number of taste receptors to be sequenced among insects. Also, it consists of 154 olfactory receptors. The olfactory and taste receptors are effective chemoreceptors which play a major role in making the organism sense its surroundings. The repellent behaviour of American cockroach can be noted by observing the movement of its antennae, labial palps and maxillary palps when exposed to organic repellents of varying concentrations. These traits enables the organism to adapt to the environment thereby enabling its survival.

It is important to study the repellent behaviour of cockroaches for the development of bioassay techniques for suitable preparation of effective organic repellents against insect pests like *Periplaneta americana*. Organic repellents are preferred over synthetic repellents for the purpose of providing protection to the user from harmful effects of synthetic repellents (Trumble, 2002). Organic repellents like clove oil and peppermint oil can also cause allergic skin reactions (Strickman,et al., 2009).Though eucalyptus oil serves as an inhalant oinment to relieve nasal congestion, it can cause Allergic Contact Dermatitis which is referred to as ACD (Kartal etal., 2016). Also, neem oil can cause skin irritation, such as dermatitis when used undiluted (Reutemann and ehrlich, 2008).

2. REVIEW OF LITERATURE

Plant-derived products serves as an effective and an eco-friendly pest control measure against insects and mites. Plant-derived products in the form of odoriferous oils serves as effective repellents against insects. The toxicity effect depends upon the concentration used (Gahukar, 2017).

Ahmad et al., (1995) evaluated essential oil extracts of six Malaysian plants for repellent activity against *Periplaneta americana*, out of which *Syzygium aromaticum* exhibited maximum repellent activity. Dose-dependent repellency ranging from 57.1 to 100 percent was reported in all six extracts when tested with the lowest concentration tested of 12 ppm.

Omara et al., (2013) studied the repellent activity of clove oil and sesame oil against American cockroach. The first nymphal stages was repelled by clove oil and sesame oil at a concentration of 2 percent and 6 percent after a 48 hour exposure respectively. All the four nymphal stages were repelled by clove oil and sesame oil at a concentration of 8 percent and 10 percent after a 24 hour and a 48 hour exposure respectively. Clove oil served as an effective fumigant whereas sesame oil didn't serve as an effective fumigant.

Clove oil contains of various compounds such as eugenol, eugenol acetate and gallic acid and it can also be used as an effective insecticide.(Cortes- Rojas et al. 2014) .

Eugenol is an active component of clove oil and exerts its insecticidal properties on octopamine receptors thereby affecting the dorsal unpaired neurons of cockroaches (Price and Berry, 2006)

Clove oil and rosemary oil can be used as an effective repellent against nymphs and adults of *Periplaneta americana* (Sharawi et al., 2013).

Sittichok and Soonwera, (2012) tested the topical application of herbal essential oils like clove oil, lemon grass oil, citronella oil, eucalyptus oil and orange oil against the nymph and adult *Periplaneta americana*. The lethal mortality values showed that lemon grass oil when along with orange oil were more toxic to the nymphs and adults of *Periplaneta americana.*

Phillips, (2009) the repellent effect of 12 essential oil components were studied on the different stages of German cockroach. Among all the essential oils, peppermint oil diluted to various concentrations showed 57 percent repellency while in the ootheca stage they exhibited 100 percent mortality.

Corn mint, *Mentha arvensis* oil in a diluted form *w*ere used as repellents against *Periplaneta americana* and *Blattella germanica.* The study reported that repellency of German cockroaches exposed to mint oil was 92.3 percent and 100 percent in case of American cockroach (Appel, et al., 2001).

Various ethanol concentrations of peppermint oil that showed high degrees of repellency against *P. americana* and *B. germanica* was reported by Thavara et tal., (2007)

The two main components of *Eucalyptus robusta* leaves extracts such as Alfa pinene and 1,8-cineole were identified by gas chromatography-mass spectroscopy method. They showed repellent activity against the nymphs of German cockroaches after one hour exposure at 5 ppm concentration (Liu et al., 2011).

Five different concentrations of eucalyptus globules were used as a repellent by filter paper and aerosol bioassay methods against *Periplaneta americana* . After 24 hours, 100 percent mortality was observed in mixed formulation (Zibae, 2015)

Various components of neem oil serves as an effective repellent against insects such as cockroaches, lice, triatomines and mosquitoes. It exhibits various modes of action affecting

the growth, fecundity and biological fitness of the organism. It also results in antifeedancy and blocks the development of vector-borne pathogens (Su and Mulla, 1999).

Mohamed *et al.,* (2014) reported that different concentrations of boric acid and neem leaflet powders can be used as a bait against *Periplaneta americana*. The powders were incorporated into food mixtures baits and the lethal dose (LD 50) was calculated for each treatment. In both the treatments, toxicity was found to be directly proportional to the amount ingested by the cockroach (mg/cockroach). Increased concentration of boric acid was found to be toxic to *Periplaneta americana* and mortality percentage increased in a positive correlation with concentration.

Rejitha, et al., (2014) studied the repellent activity of plant powder of *Azadirachta indica*, *Curcuma longa*, *Vitex negundo, Adhathoda vasica* and *Ocimum tenuiflorum* against *p.americana.* In all the experimented plants maximum repellency occurred at the highest concentration.

Azadirachtin, an active component of neem leaves exerts an excitatory action in the nervous system of cockroach by interfering with the ion channels in the nerve membrane, the probable target of several insecticides (Shafeek et tal., 2004)

Tichy et al., (2005) reported that a morphologically identifiable type of olfactory sensillum was present on the antennae of cockroaches which consisted of a pair of ON and OFF cells. These cells were capable of detecting the various concentrations of fruit odour.

Trumble, (2002) reported that plant-based repellents are safer than DEET because they are natural. However, some natural repellents are safer than others, which cannot be equated to safe.

The American cockroach along with its mouthparts makes use of its foreleg tibia and tarsus to get hold of its antennae and bends it into a loop while cleaning its antennae from the base to the tip (Robinson, 1996).

The antennal hair-plates of *Periplaneta americana* can be used in object-guided tactile orientation of the cockroach which makes it possible to orient itself in a particular direction and angle (0^{o},45^{o} and 90^{o}) when it encounters an obstacle. The cockroach horizontally spans the surface of the obstacle using its antennae. The presence of mechanosensitive hairs on the basal segment of the antennae are responsible for this behaviour (Okada and Toh, 2000).

According to Prakash et al., (1995) the maxillary palp of *Periplaneta americana* has a long, slit like micro-furrow present on ventral side of the medial surface of the fifth segment densely populated with numerous sensilla consisting of numerous grooves along the hair shaft and a slit near the distal tip. The sensilla is about 3 to5µm high and about 1.5 to 1.8µm in thickness. GAS sensilla is the type of sensilla responsible for olfaction present in the maxillary palp of *Periplaneta americana*.

The first segment of the joints of maxillary palp of *Blattella germanica* was more sensitive to pressure exerted on the cuticular skeleton and passive straight or sideways bending of the joints. It was excited to a lesser extent when the segment was actively moved by its own muscles which was possible due to the presence of campaniform sensilla on the joints of the palps of the insect (Ramaswamy and Gupta, 1981).

According to Chapman, (1965) the spines present on the legs of *Periplaneta americana* consists of numerous campaniform sensilla, mechanosensory in function which aids in tactile orientation of the cockroach.

3. AIM AND OBJECTIVE

In the present study the efficacy of odoriferous oil viz; Eucalyptus oil,clove oil,neem oil, peppermint oil as effective repellant against *p.americana* was undertaken . Ad libitum sampling methods were employed.

The aim of the present study

- To understsnd the repellant behaviour of *p.americana* against the odoriferous oils
- To test the repellant efficacy of the odoriferous oils.

4. MATERIALS AND METHOD

4.1 Experimental setup

The study was conducted in the laboratory of the department of Advanced Zoology and Biotechnology, Women's Christian College under optimum temperature and humidity of 29°C and 75 percent respectively. A rectangular box measuring about 30 cm and 21 cm in length and breadth was used to assess the repellent activity of *Periplaneta americana* The odoriferous oils used for the study were clove oil, neem oil, eucalyptus oil and peppermint oil and liquid paraffin was used as a dilution factor . Dilutions such as 1:50,1:10,1:2 and 1:0 of varying concentrations were used respectively. For the present study, 40 cockroaches were used and each were subjected to 10 trials for 4 different odoriferous oils. The cockroaches were introduced one at a time into the transparent rectangular box and it was then covered using a transparent sheet.A drop of the test solution was placed on a filter paper and was introduced into the box through a small, rectangular outlet present on one side of the container. The repellent of the cockroach was then observed. After exposing the cockroach to varying concentrations, a time interval of 30 minutes was given before the cockroach was shifted to another container for a subsequent trial with a different concentration.

4.2 Preparation of test concentrations of odoriferous oils

Preparation of 0.5% concentration of the test solution:

1 drop of respective odoriferous oils which includes clove oil, eucalyptus oil, neem oil and peppermint oil followed by 49 drops of dilution factor i.e, liquid paraffin was used in the preparation of 1:50 dilution i.e, 0.5% concentration of the test solution**.**

Preparation of 10% concentration of the test solution

1 drop of respective odoriferous oil followed by 9 drops of liquid paraffin was used in the preparation of 1:10 dilution i.e, 10% concentration of the test solution.

Preparation of 50% concentration of the test solution

1 drop of respective odoriferous oil followed by 2 drops of liquid paraffin was used in the preparation of 1:2 dilution i.e, 50% concentration of the test solution.

Preparation of 100% concentration of the test solution:

1 drop of respective odoriferous oil alone was used in the preparation of 100% concentration of the test solution.

4.3 Test organism

The adult cockroaches of *Periplanetaamericana* that were collected from drains, crevices of doors and various other dark and moist habitats and were reared in separate rectangular containers with perforated lid in order to provide aeration necessary for its survival. The cockroaches were also provided with water and sugar up until half an hour before the experiment was conducted.

5. OBSERVATION AND RESULTS

5:1 Repellent efficacy of the odoriferous oil

All the cockroaches that were subjected to test solutions of varying concentrations showed contact irritancy.The repellant behaviour of *P. americana* against the four odoriferous oils are represented in table 1.When the concentration of test solution was 0.5 percent with a dilution factor of 1:50, the cockroach took the longest time to get repelled in case of peppermint oil the range lies between 200to250 seconds followed by neem oil having its repellency rate lying in between 50 to100seconds.The time taken to be repelled by eucalyptus oil lies within the range of 65to75 seconds and in case of clove oil it lies within the range of 50 to 60 seconds. From this we can say that, test solutions with the highest dilution factor are directly proportional to the repellency rate of the cockroach(Fig.1).

The repellency rate gradually decreased as the concentration of the test solution increased i.e, repellency rate of the cockroach is inversely proportional to the concentration of the test solution. When the concentration of the test solution was 10 percent with a dilution factor of 1:10,the repellency rate was relatively higher than in the case of test solution with a dilution factor of 1:50.The time taken for the cockroach to be repelled by peppermint oil lies within the range of 150 to 200 seconds,60 to 70 seconds in case of neem oil,20 to 50 seconds in case of eucalyptus oil and 15 to 25 seconds in case of clove oil(Fig.2).

When the concentration of test solution was 50 percent with a dilution factor of 1:2,the repellency rate lied within the range of 100 to 150 seconds in case of peppermint oil,40 to 50 seconds in case of neem oil,10 to 20 seconds in case of eucalyptus oil and 5 to 10 seconds in case of clove oil(Fig.3).

The highest repellency activity was observed in the clove oil followed by eucalyptus oil, neem oil and peppermint oil when the concentration of the test solutions were 100percent.The cockroach showed Repellant behaviour within the range of 1 to 5 seconds when subjected to clove oil. In case of eucalyptus oil the repellency behaviour was observed within the range of 5 to 10seconds, 30 to 40 seconds in case of neem oil and 50 to 100 seconds in case of peppermint oil. Thus, the test solutions which are in their most concentrated form are inversely proportional to repellency rate of the cockroach(Fig.8).

5.2 Repellant behaviour of *periplaneta americana.*

The American cockroach cleans its antennae from the base to the tip using its mandibles. The cockroach gets hold of its movable antennae also known as antennal flagella using its shortest prothoracic legs and when exposed to any physical barrier it immediately moves it legs which indicates that the cockroach is sensitive to touch .This is possible only due to the presence of campaniform sensilla which are tactile in function. It also curves its antennae into a loop using its prothoracic legs during the process of cleaning from the base to the tip. The segmented antennae is shorter and thicker at the base and is longer and thinner towards the tip. The antennal grooming behaviour is of great importance because of the major role it plays in sensing the smell of its surroundings and the blockage of the cuticular pores makes the organism incapable of sensing danger in the form of harmful environmental pollutants and also hinders it from finding food and a suitable mate due to its inability to detect the smell of food and sex pheromones. The cockroach wriggles its maxillary palp even before directly coming into contact with the repellent. This shows that the maxillary palp is also olfactory in function due to the presence of GAS sensillum on its surface. Blocking the olfactory receptors of the insect's antennae and maxillary palp is crucial in developing effective repellents of suitable concenctrations. The cockroach when encounters an obstacle or any physical barrier is able to turn away from the obstacle in a

particular angle and orient itself in a particular direction. This is possible due to the presence of antennal hair-plates on the antennae and campaniform sensilla present on the maxillary palp and legs of the cockroach which are in the form of spines. It horizontally spans the surface of the obstacle using its antennae which is crucial in tactile orientation of the cockroach . The primary function of the maxillary palp is gustation for it uses its palps for feeding purposes and is essential for sensing harmful environmental pollutants. Thus the olfactory, tactile and taste receptors of *Periplaneta americana* play a very important role in performing the act of getting repelled when subjected to organic repellents of varying concentrations. The extent of getting repelled depends upon the concentration as well as the chemical composition of the repellent itself.

6.DISCUSSION

According to the findings of the study, the repellency rate of *Periplanetaamericana* was the lowest when the cockroach was exposed to peppermint oil of varying concentrations. This indicates that the repellent activity of peppermint oil is not strong enough to repel the insect instantly when exposed to the repellent. Phillips, (2009) concluded that peppermint oil exhibited least toxicity and repellent activity which is similiar to the findings of the study.whereas Thavara et tal.,(2007) reported that *periplaneta americana* exhibited 95 percent repellency when subjected to peppermint oil where ethanol is used as dilution factor.

When *P.americana* was exposed to peppermint oil of varying concentrations, it took the longest time period to be repelled by the test solution when compared to other test solutions of varying concentrations. Based on the results obtained, it can be seen that *P.americana* exhibited least repellency.On the contrary, Corn mint oil showed 100% repellency in case of adult American cockroaches (Appel et tsl.,2001)

P.Americana overall exhibited highest repellency activity when subjected to clove oil of varying concentrations. This indicates that least time was taken for the cockroach to be repelled by clove oil .

6.1 Repellant efficacy of odoriferous oils

Plant derived products in the form of essential oils serve as effective repellant against insects and their toxicity effect depends upon the concentration used(Gahukar,2017).

In the present study ,*periplaneta americana* showed immediate repellant behaviour within 1to5seconds when exposed to clove oil at 100 percent concentration. Clove oil can be used as an effective repellant against both the nymphs and adults of *periplaneta americana*

(Philip,2009 ;Sittichok and soonwera; 2012,sharawi et.al.,2013).clove oil contains secondary metabolites like eugenol, eugenol acetate and gallic acid which can be used as an effective insecticide(Cortis-Rojas et. al., 2014).price and berry, (2006) reported that eugenol exerts its insecticidal properties on the octopamine receptors thereby affecting the dorsal unpaired neurons of cockroaches.

On exposure to eucalyptus oil ,cockroaches exhibited repellant behaviour is 5 to 10seconds. Liu et.al.,(2011) reported that two components alfa pinene and 1,8-cineole identified by GCMS analysis showed repellant activity against the nymph of German cockroaches after one hour exposure to 5ppm concentration. 5 different concentrations of eucalyptus globules were used as repellants against *periplaneta americana* and it was observed that they exhibited 100percent mortality (Zibaee, 2015).

Periplaneta americana Exhibited repellant behaviour in about 30 to40seconds when exposed to neem oil.Su and Mulla, (1999)reported that neem oil serve as an effective repellant against cockroaches and that they act as antifeedant and exhibit various modes of action affecting the growth, fecundity and biological fitness of the organism. Azadirachta indica plant powders exhibited maximum repellancy against *periplaneta americana* at highest concentrations(Mohamed et al.,2014,Rejitha et al., 2014.Shafeek et al., (2004) reported that Azadirachta an active compound of neem leaves exerts an excitary action in the nervous system of cockroach by interfering with the ion channels in the nerve membrane.

Periplaneta americana exhibited least repellant behaviour of 50to100seconds exposed to peppermint oil .Phillips, (2009) reported that peppermint oil exhibited only 57.5percent repellancy against the adult cockroaches whereas 100percent mortality was Obseved in the ootheca stage. Whereas Thavara et al., (2007) reported that *Periplaneta americana* exhibited

95percent repellant activity when subjected to peppermint oil when ethanol is used as a dilution factor.

6.2 Repellant behaviour of *periplaneta americana*

The antennal grooming behaviour from the base to the tip using its mandibles was observed in *P.americana*. The cockroach made use of its small forelegs to get hold of its antennae and bent it into a curved loop when it was subjected to repellents of varying concentrations. This behaviour of *P.americana* is of great significance for it aids in olfaction. The findings of the study were similiar to the findings of Robinson, (1996) who worked on antennal grooming behaviour of *Blattella germanica.* The antennae of *P.americana* is olfactory in function this is because all the cockroaches that used its antennae to sense the smell of the repellent delivered on the filter paper retreated.

The presence of mechanosensitive hairs on the basal segment of the antennal hair-plates played an important role in tactile orientation behaviour. The cockroach was able to orient itself in a particular direction and turned away from the filter paper coated with repellent which served as an obstacle by horizontally spanning the filter paper using its antennae.The findings of the study were similiar to the observation of Okada, 2000 who worked on object-guided tactile orientation behaviour of *P.americana* where metal pole was the object used.

The American cockroach began to wriggle its maxillary palp when subjected to repellents of varying concentrations. This action was performed when the maxillary palp was not in direct contact with the filter paper onto which the test solution was delivered. This indicates that the maxillary palp is not only gustatory or mechanosensory, but is also olfactory in function. According to Prakash, et tal.,1995 the maxillary palp of *P.americana* has a long, slit like micro-furrow present on ventral side of the medial surface of the fifth

segment densely populated with numerous sensilla consisting of numerous grooves along the hair shaft and a slit near the distal tip. The sensilla is about 3to5µm high and about 1.5 to 1.8µm in thickness.GAS sensilla is the type of sensilla responsible for olfaction present in the maxillary palp of *P. americana.*

The presence of numerous tiny hairs present on the first segment of the joints of maxillary palp of *P. americana* were tactile function. This was evident when the maxillary palp of the cockroach came in contact with filter paper onto which the repellent was delivered. According to Ramaswamy and gupta,(1981) the campaniform sensilla present on the first segment of the joints of maxillary palp of *B.germanica* was tactile in function and was able to detect the presence of any obstacle on its way. The sensilla was excited only to a lesser extent when the segment was actively moved by its own muscles and responded to passive straight or sideways bending of the joints and strongly to pressure on the cuticular skeleton of the insect.

Numerous tiny spines on the legs of *P.americana* were observed which inturn consists of numerous tiny sensilla referred to as campaniformsensilla. When the cockroach came in contact with the filter paper onto which the repellent was delivered, it moved away. This indicates that the legs of *Periplanetaamericana*were tactile in function. According to Chapman, (1965) histological sections of the leg of *P.americana* reveals the presence of a single campaniformsensillum in the thick cuticular walls of the spine mechanosensory in function.

7.SUMMARY

- The adult cockroaches of *Periplanetaamericana* were collected from drains, crevices of doors and various other dark and moist habitats.
- It was then reared in separate rectangular containers with a perforated lid inorder to provide aeration necessary for its survival.
- The various types of odoriferous oils used for the study were eucalyptus oil, neem oil, clove oil and peppermint oil.Various concentration of 1:50, 1:10,1:2,1:0 the odoriferous oil was preparded by using liquid paraffin as dilution factor.
- Ten trials were performed using ten cockroaches for each odoriferous oils Highest repellency was observed in case of clove oil and lowest repellency was observed in case of peppermint oil.Repellency rate is directly proportional to the concentration used.
- The extent of the insect getting repelled depends upon the concentration as well as the chemical composition of the repellent.
- The American cockroach uses its gustatatory, olfactory and tactile receptors to sense certain environmental cues to protect itself from danger.The repellent behaviour of *P. americana* such as antennal grooming behaviour, object-guided tactile orientation behaviour and gustation using various sense organs which includes the antennae, maxillary palp and legs of the cockroach is of great significance in the preparation of suitable organic repellents of varying concentrations against this insect pest.
- The preference of organic repellents over synthetic repellents is for the purpose of providing protection to the user from harmful effects of synthetic repellents.