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Neo-simple methodology for the evaluation of potential botanical insect repellents and the rapid comparative study on specific chemical and photo sensitivity of selected insects

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ABSTRACT

The study was to evaluate the insect repellent activity of certain plants and to compare the sensitivity of certain insects towards selected essential oils, composite light and a few colors.

While on the experimental arena, chemical and photo sensitivity tests were carried out using a 'modified T-maze' called as 'A-B sensitivity apparatus'. Plant parts were extracted using methanol-water and ethyl acetate. Essential oil was extracted with hydro distillation method. Preliminary evaluation procedure was focused on repellent activity against *Sitophilus granarius*, but further studies used more insect species. Using new indices, the repellent and insect potential were compared.

In Preliminary evaluation, 18 out of 19 plants showed repellent activity. Further studies on the essential oil of available plants showed that, *Merremia vitifolia* has the highest repellent activity than the Peperomia Pellucida and the positive control, *Elettaria cardamomum*. *Merremia vitifolia* essential oil have the lowest absolute effective surface concentration and highest repellent index. Considering the Insect properties, *Sitophilus granarius* is the strongest and hence had the highest Anti-Repellent Index of 5.8421 µl/gcm^2 against *Elettaria cardamomum* essential oil. In photo sensitivity test all the insects showed more affinity towards the middle of the visible spectrum and are repelled by darkness.

Keywords: Insect repellent, Chemical sensitivity, Photo sensitivity, Absolute effective surface concentration, Anti-repellent Index.

INTRODUCTION

Beneficial or non-pest insects comprise 99.5% of the total number of the known insect species. Of the remaining 0.5%, only a few of these can be a serious menace to people. But these few can cause more damage annually than destruction of one fifth of the world's total crop production (Sallam, 1999) and 17% of all infectious diseases which cause more than 1 million deaths (WHO, 2017). Hence efficient pest management is essential.

While the control of insects by chemical pesticides have proven negative effect, which is catastrophic to humans and the environment. Pesticides are responsible for an estimated 200,000 acute poisoning deaths each year and destruction of the ecosystem. But this anti-life challenge of chemical pesticides has been exacerbated by a systematic denial, fueled by the pesticide and agro-industry, of the magnitude of the damage inflicted by these chemicals, and aggressive, unethical marketing tactics remain unchallenged (UN General Assembly, 2017). In contrast to chemical pesticides, the botanical pesticides have Reduced toxicity to non-target organisms, reduced persistence in the environment, usable in organic agriculture, Low mammalian toxicity and Safe for farm-workers and nearby residents (Seiber *et al,* 2014). The use of botanicals against insect dates back to Rigvedic era (Matthews and Matthews, 2010), and ancient References to plant protection were found even in Vedas, Krishiparashara, Sangam literature of Tamil, Agnipurana, Brhat Samhita, Vrikshayurveda, etc (Nene, 2003).

As nature being an almost unlimited source of bio-active natural products with pesticide activity, the current model of relying almost exclusively on synthetic chemi-cals as a source of pesticides is beginning to change. Also, many government policies like the new European legislation for the registration of plant protection products encouraging the development of less harmful substances, is causing natural products to begin to replace traditional pesticides, or provide the basis for the synthesis of new ones (Villaverde *et al*. 2016).

Botanical repellents are even more effective in protecting the non-target organisms, insect predators and environment. Moreover, insects become resistant to an insecticide more quickly than a repellent. New researchers point out the use of different bio-pesticide, repellents and integrated pest management for an effective and enduring pest management (Seiber *et al,* 2014; Isman, 2006; Lynch and Boots, 2016). For that, reliable evaluation of insect sensitivity and behavior is necessary.

Repellent activity of *Elletaria cardamomum* (L.) *Maton* essential oil is known, for eg, 1µl dose of *Elletaria cardamomum* (L.) *Maton* essential oil showed repellent activity of 38% against *Lasioderma serricorne* in olfactometer method (Hori, 2003). Granary weevil is chemical-sensitive and both sexes showed similar responses to different blends of cereal volatiles (Piesik and Wenda-Piesik, 2015). So *Elletaria cardamomum* (L.) *Maton* essential oil was used as a positive control and Granary weevil as the primary test insect.

Popular methods to determine insect's repellent activity were area preference method and Y-tube olfactometer method. The current area preference method is not reliable for volatile compounds. In a repellent activity test against *Sitophilus granarius* using area preference method, 0.1% of essential oil of *Hyptis suaveolens* showed 42.5% repellent activity while 1% showed only 17.5%. Similarly, the repellent activity isn't directly proportional to the concentration of pure compounds like Sabinene, Limonene, etc. Also, there has been inconsistency in repellent activity during different time intervals. The results of Pitfall bio-assays are also not different from the general trends of area preference method results. Similarly, there were inconsistency in the results of different research articles (Benelli *et al,* 2012). Y-tube olfactometer uses an airflow, so eludes the effect of default experimental condition of fundamental/ native diffusion. Similarly, comparisons of the results were also difficult due to the variability on the methodologies, conditions found in literatures (Nerio *et al,* 2010) and lack of appropriate indices. So fundamental issues need attention.

In the physical world, diffusion is one of the basic property of volatile compounds and spatial repellents. The Einstein–Smoluchowski equation is the central connection between the microscopic details of particle motion and the macroscopic parameters relating to diffusion. So, diffusion coefficient(D) is given by the formula.

$$D = \frac{\lambda^2}{2\tau}$$

Where λ (lambda) is the length of each step (which in the model is assumed to be the same for each step) and τ (tau) is the time each step takes. This equation tells us that a molecule that takes rapid, long steps has a high diffusion coefficient λ (lambda) is the length of each step (which in the model is assumed to be the same for each step) and τ (tau) is the time each step takes. This equation tells us that a molecule that takes rapid, long steps has a high diffusion coefficient (Peter and de Paula. 2010). The diffusion coefficient varies between molecules and Methane has the maximum reported D of 173 at 25°C (Tang *et al.,* 2015).

Insects were sensitive to light also. The light traps used for controlling pests are the testament of this technology (Shimoda, 2013) forcing the need for analyzing photo-sensitivity of insects in laboratory conditions.

Table 1: Maximum possible distance traveled by Gas Molecule (25ºC)

Molecule	*Diffusion coefficient (25°C)*	*Distance(cm) traveled by molecule in time(s)*									
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Methane	173	18.60	26.31	32.22	37.20	41.59	45.56	49.21	52.61	55.80	58.82
Methanol	137	16.55	23.41	28.67	33.11	37.01	40.55	43.79	46.82	49.66	52.35
Formic acid	120	15.49	21.91	26.83	30.98	34.64	37.95	40.99	43.82	46.48	48.99
Propene	110	14.83	20.98	25.69	29.66	33.17	36.33	39.24	41.95	44.50	46.90
Methyl acetate	95	13.78	19.49	23.87	27.57	30.82	33.76	36.47	38.99	41.35	43.59
Benzene	75	12.25	17.32	21.21	24.49	27.39	30.00	32.40	34.64	36.74	38.73
Anthracene	44	9.38	13.27	16.25	18.76	20.98	22.98	24.82	26.53	28.14	29.66
Diethyl phthalate	42	9.17	12.96	15.87	18.33	20.49	22.45	24.25	25.92	27.50	28.98
Di-n-butyl phthalate	27	7.35	10.39	12.73	14.70	16.43	18.00	19.44	20.78	22.05	23.24

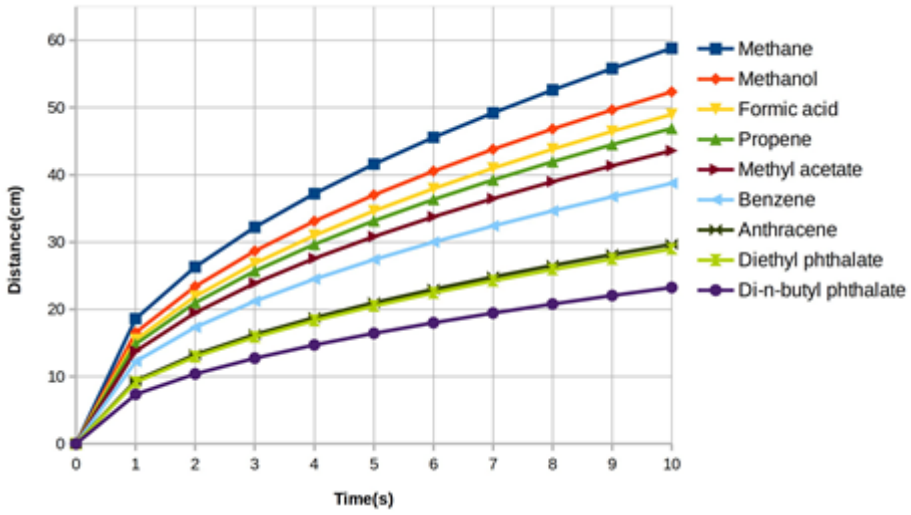


Figure 1 : Maximum possible distance traveled by Gas Molecule (25ºC)

The objectives of current study is to find new insect repellents or attractants based on botanicals and/or lights. And also to find better methodologies to determine and compare the chemical and photo sensitivity of insects.

MATERIALS AND METHODS

2.1 Collection of plant materials

For preliminary evaluation, 19 plant species were used. Among them, *Podocarpous gracilior*, *Pithecellobium dulce*, *Cassalia curviflora*, *Dryneria quercifolia* and *Tectonia grandis* were collected from the campus of St. Thomas College, Pala. Other fourteen plants were collected from accessible barren uncultivated lands, and land near sacred groves in the Kottayam, Ernakulam and Pathanamthitta districts of Kerala. Insecticidal property and repellent activity of *Elletaria cardamom* is well known (Singh, 2014) so, *Elletaria cardamomum* essential oil was used only for advanced analysis. While *Merremia vitifolia* and *Peperomia pellucida* are used for both preliminary evaluation and further studies. Both seeds and leafs of *Hydnocarpus laurifolia* and *Tabernaemontana alternifolia* were separately analysed. Details of plants used for preliminary evaluation and further studies are illustrated in table 2.

Table 2: Details of plants used for preliminary evaluation and further studies.

S. no.	*Scientific name*	*Group*	*Family*	*Parts used*	*Extract analyzed*
1	*Averrhoa bilimbi* Linn.	Dicot	Oxalidaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
2	*Cosmos sulphureus* Cav.	Dicot	Asteraceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
3	Chassalia curviflora (Wallich) Thwaites	Dicot	Rubiaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
4	*Cinnamomum malabatrum* (Burm.f.) Bl.	Dicot	Lauraceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate. 3. Essential oil.
5	Corypha umbraculifera L.	Monocot	Arecaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
6	*Cuscuta reflexa* Roxb.	Dicot	Cuscutaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
7	*Drynaria quercifolia* (L.)	Fern / Pteridophyta	Polypodiaceae	Rhizomes	1. Methanol-Water 2. Ethyl acetate.
8	*Hydnocarpus laurifolia* (Dennst.) Sleumer.	Dicot	Achariaceae	1. Buds with tender leafs and stem. 2. Seeds.	1. Methanol-Water 2. Ethyl acetate.
9	*Dendrophthoe falcata* (L.f.) Ettingsh	Dicot	Loranthaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
10	*Merremia vitifolia* (Burm.f.) Hallier f.	Dicot	Convolvulaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate. 3. Essential oil.
11	*Mikania scandens* B.L.Rob.	Dicot	Asteraceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate. 3. Essential oil.
12	*Mimosa diplotricha* C. Wright ex Sauvalle	Dicot	Fabaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
13	*Pajanelia longifolia* (Willd.) K.Schum	Dicot	Bignoniaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate. 3. Essential oil.
14	*Peperomia pellucida* Kunth.	Dicot	Piperaceae	Shoot	1. Methanol-Water 2. Ethyl acetate. 3. Essential oil.
15	*Pithecellobium dulce* (Roxb.) Benth.	Dicot	Fabaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
16	*Afrocarpus gracilior* (Pilg.) C.N.Page	Pinophyta / conifers	Podocarpaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate. 3. Essential oil.
17	*Sarcostigma kleinii* Wight & Arn.	Dicot	Icacinaceae	Leaf	1. Methanol-Water 2. Ethyl acetate.
18	*Tabernaemontana alternifolia* L.	Dicot	Apocynaceae	1. Buds with tender leafs and stem. 2. Seeds.	1. Methanol-Water 2. Ethyl acetate.
19	*Tectona grandis* L.f.	Dicot	Lamiaceae	Buds with tender leafs and stem.	1. Methanol-Water 2. Ethyl acetate.
20	*Elletaria cardamomum* (L.) Maton	Monocot	Zingiberaceae	Seeds	1. Essential oil.

2.2 Insects

For rearing *Sitophilus granarius*, one liter transparent and cylindrical PET jar with about 9cm diameter and air tight screw cap was used. In the center of the lid a square hole with length of approximately 40% of lid diameter is made. To fill the square hole, a stainless steel square Mesh (mesh no.120) and having a length of about 1cm bigger than that of the hole length was used. It was tightly fixed to the outside surface of the lid using epoxy adhesive. Sterilized whole wheat was used for rearing *S. granarius*. It was sterilized by heating in a hot air oven to 60°C for 15 minutes (Iowa State University, 2017).

Initial specimen of *Sitophilus granarius* was collected from market sourced infested wheat. Ten active adults were transferred to jar with 50 gram of wheat. After three weeks, the introduced adults are removed from the jar. When new adults emerge, Active and healthy insects were transferred to another jar containing 50g of wheat. These new adults were removed and discarded after three weeks. Similarly, another generation of adults were also removed and discarded. Then the remaining wheat in the jar containing eggs of the third generation were used for maintaining the culture.

For maintaining the culture, the top quarter of the infested wheat from the jar in which third or later generation insects or eggs were present was transferred to another jar and then 50 grams of sterilized wheat was added to the jar. There after culture is maintained by adding 75 grams of sterilized wheat every week up to one month. Then top quarter of the infested wheat and needed insects were transferred to a new jar. Another quarter from the top can also be used to inoculate another jar. Or if the insect population is much higher or more replicates were needed, 20 active adults alone can be transferred to another jar. Then 50g of sterilized wheat was added to each jar. Only excess adults were discarded. There after culture was maintained by adding 75 grams of new sterilized wheat every week up to one month. The above process is repeated to maintaining and/or multiplying the culture. The culture was maintained at 26±2 °C and 75±5% humidity.

Adults of *Henosepilachna vigintioctopunctata* (Hadda beetle) and Pyrrhocoridae family were collected from infested bitter gourd and seed-head of matured Sorghum plants respectively. Plants were grown in the agricultural land with-out insecticide application.

In all the cases active adult insects of all sexes were selected for analysis.

2.3 Plant tissue extraction

Fresh plant tissues other than seeds were cut in to small pieces with knife or scissor, while seeds and small rhizomes pieces were crushed with mortar and pistil. 10 gram of this tissue were weighted and homogenized for 5 minute in an electric mixer with 100 ml Methanol-Water (4:1) solvent mixture. The homogenized material was filtered using grade1 filter paper. The filtrate was evaporated in room condition to about 70ml and if necessary the evaporated filtrate was made up to 70ml using methanol-water (4:1) solvent. And this filtrate was used as methanol-water extract.

The residue obtained was kept in capped conical flask with 70ml ethyl acetate and gave mild intermittent shake for 24 hours and filtered. This filtrate was made up to 70ml using ethyl acetate and used as ethyl acetate extract (Harborne, 1998).

Essential oil is extracted by hydro-distillation using clevenger apparatus. 300gm of cut or crushed fresh plant tissue or 100gm of crushed seed and 300ml of distilled water were added to 1000ml flat/round Bottom flask and distilled (Drew, 2012).

For preliminary evaluation purpose 3% v/v essential oil in acetone was used, while for advanced analysis undiluted essential oil was used.

2.4 A-B sensitivity apparatus construction

A-B sensitivity apparatus is a modified T-maze, with a transparent tube having two sides or choices and an insect introduction tube inserted in the middle.

2.4.1 Main Tube

The main part of the instrument is a thick transparent/clear PVC tube called main tube, having one inch (2.54 cm) inner diameter and 105cm length. An exact hole is made in the exact center (52.5cm from ends), on the upper surface of the tube, so that a half inch nylon hose connector can be inserted. The hole was made using a half inch metal hose connector. The metal connector was moderately heated and was gently pressed in the marked central position. The process was repeated till an exact hole was melted out. Care was taken by controlling the excess heat from melting a bigger hole.

For photo sensitivity assay, a shorter main tube having a length of 40cm was used.

2.4.2 Insect introduction Tube (IiT) and IiT closer

2.4.2.1 Insect introduction Tube (IiT):

A half inch hose connector was cut into exactly 2 half to reduce its length to nearly 4.2cm. This half connector was inserted in to the hole in the main tube, so that the uncut face is facing the inside of the tube. The tube is inserted to a length, till the first grove of the connector attaches to the inner side of the tube. Nearly 0.5cm of the connector is projected inside the tube. Thus half of the half inch hose connector becomes IiT.

2.4.2.2 IiT closer:

Its a solid rod with a diameter slightly less than of the inner diameter of the IiT and length higher than that of IiT. Here a new pencil piece with nearly 5.5cm length was used. At one end of the pencil piece, adhesive tape were wound to increase its diameter so that it is only slightly less than the inner diameter of IiT. On the other end the length above 4.2cm is thickened with adhesive tape to a diameter higher than the outside diameter of the IiT. So one end of the IiT closer, covers the inside end of the IiT and stays at the same level. Inside the main tube, IiT closer and IiT had the same length. Therefore, the insect does not come out of the apparatus through IiT. Also the diffusion from outside the main tube through the IiT will be minimized.

2.4.3 Main Tube base

Two ½ or ¾ inch dull white colored pvc pipe of 105cm length were placed on the underside of the main tube and tied using moderately tight rubber band to prevent unwanted movement of the tube and to keep the tube straight. In chemical sensitivity test, for better visibility white paper strip with a width of 5cm can be placed in between the main tube and main tube base with its edges bending downwards. See figure2(d).

For photo sensitivity assay, a shorter base having a total length of 35cm was used.

2.4.4 Choice sides

For the choice ends, a one inch hose connector was cut in to two half of nearly 6.5 cm length. Material selected was pure cotton white cloth with a thread count of 200. Square pieces having length 6.5cm were cut and used for the assay. One of the one inch connector half and 2 pieces of cloth were used to form one choice side. Two pieces of cloth were used to prevent the insect from piercing and escaping through the ends. The assembled and dissembled condition of the short version of A-B sensitivity apparatus is shown in Figure 2.

2.4.5 Distraction reduction arrangement

The apparatus should be placed in a place where there is clean air, 25°C temperature, 75±5% humidity, uniform light, devoid of excess air flow and direct or harsh light. Also, to support the arrangement, the apparatus is kept in a box, so that apparatus is exposed to only uniform soft light and minimum external airflow, so that distraction to the insect or test setup can be reduced. The apparatus was kept in marked positions.

As only one test can be observed at a time, a rectangular box with a dimension of about 135cm length, 25 cm width and 25cm height was used for chemical sensitivity test. A box of 70cm square base and 30 cm height was used for photo sensitivity test, so that six replicates can be examined at a time. Only Top/front side of the box was opened. For having easy accesses to the test and control side during chemical sensitivity test, a 6cm square openings at the same plane of the apparatus with a black paper lid is made on both short sides.

The inside of the box was covered with non-reflective black/dark gray colored paper. The purpose of this is to minimize the interference of light and to force the insect to focus the white end sides.

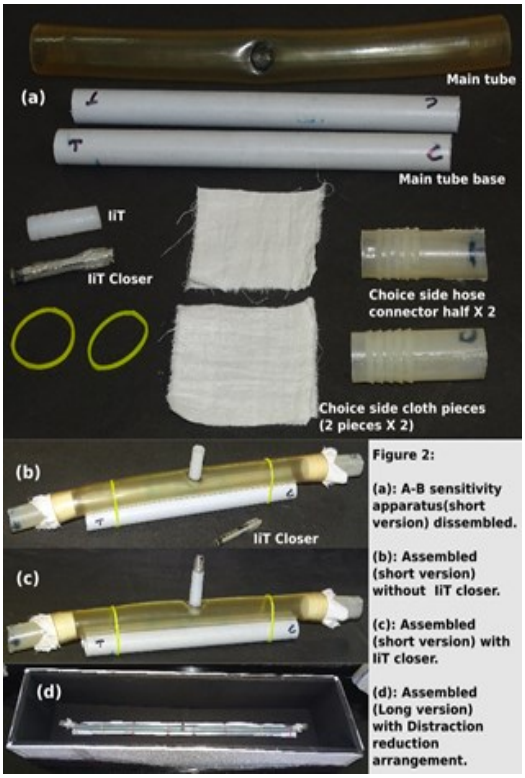
2.4.6 For photo sensitivity test.

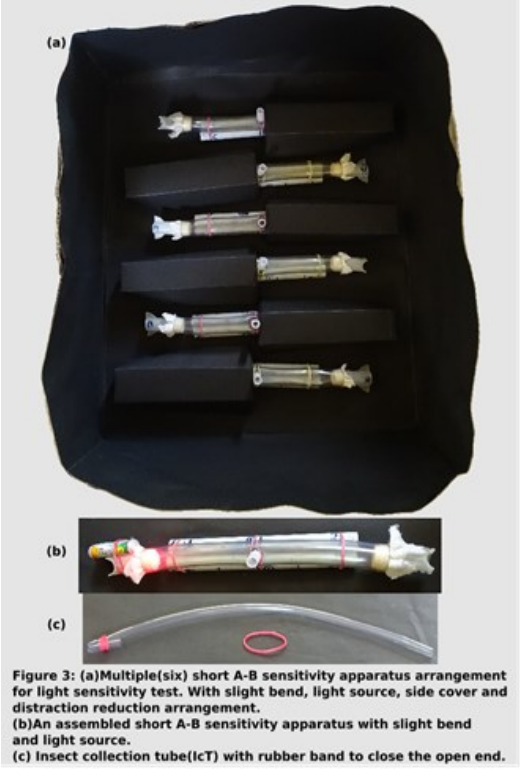
2.4.6.1 Light source:

The light sources were 2.5V-3V LED connected to 2AA Batteries. 2 Batteries and a LED of required color is connected in serial. Two batteries were connected in serial using an appropriate dual battery holder. And LED is connected to battery holder using insulated wires having minimum length. Non-conductive sleeves were placed in each leg of LED to prevent exposed metal parts from short circuit. LEDs of Blue, Green, Yellow and Red colors were used.

2.4.6.2 Side Cover:

In order to prevent/reduce composite light entry into test side, the test side is covered by a rectangular box made of thick non-reflective black paper. The box has a length of 28cm, while width and height are 8cm. One short and one long side of the box were removed for easy handling. The open short side came at the IiT side and the open long side faces the bottom.





2.5 Insect preparation and Insect introduction

2.5.1 Insect preparation:

Only *Sitophilus granarius* was used for preliminary evaluation, but for advanced studies and photo-sensitivity tests *S. granarius, H.vigintioctopunctata* adult, *H.vigintioctopunctata grub* and insects of Pyrrhocoridae family were used. Ten adults of *S. granarius,* six adults of *H.vigintioctopunctata* and Pyrrhocoridae family and six grubs of *H.vigintioctopunctata* were used for their test.

Just before the test, ten active adult insects of *Sitophilus granarius* were captured in to a ¼ inch transparent tube of 30 cm length, whose bottom end was tightly folded using a rubber band. After putting all the ten insects in to the tube, the top end was also tightly folded with a rubber band. The tube is called Insect Collection Tube (IcT). See Figure3. While for *Henosepilachna vigintioctopunctata* and Pyrrhocoridae family, active adults were gently captured and kept inside petridishes.

2.5.2 Insect introduction:

In the advanced analysis, the repellent potential of the pure volatile oil has to be analyzed, so the insects were introduced before the volatile compound reaches the point of introduction of the insect. As the volatile compounds diffuses inside the A-B sensitivity apparatus quickly and the spatial concentration increases to certain differentiation/saturation limit, the insect may become incompetent to discriminate the concentration gradient. Therefore, the insects were introduced before the compound with the best diffusion coefficient diffuses and reaches the insect introduction point from the test side. So, in advanced analysis, insects were introduced within 6 seconds after the application of essential oil to the test side.

While for preliminary evaluation and photo sensitivity test, it was within 10 seconds after the completion of test side assembly. Because, the presence of volatile compounds in the test cloth piece used for preliminary evaluation were considerably reduced during the process for the evaporation of solvents. So that the chances that the volume concentration of the quickly diffusing volatile compounds inside the A-B sensitivity apparatus to rise above the differentiation/saturation concentration limit of the insect is virtually impossible. In the case of photo sensitivity test, there isn't any involvement of any such compounds.

For adding *S. granarius* in to main tube for testing, one end of IcT with ten adult insects of *S. granarius* was opened and the opened end was inserted deep into the IiT so that the end almost touches the bottom side of the main tube. To aid the insect introduction, IcT was gently knocked with fingers. After that IcT was removed from the IiT. Then IiT closer was placed in the IiT. In the case of insects other than *S. granarius,* they were gently hand-

picked and added into the IiT. After adding the needed insects, IiT closer is placed in the IiT. Insects were discarded after the test.

2.6 A-B sensitivity apparatus Setup

2.6.1 Chemical sensitivity assay:

The test was conducted only with clean and dry apparatus. Apparatus was kept in horizontal position, either 'Control' end on the left side and 'Test' end on the right or vice versa. Required distraction reduction arrangement (*section 2.4.5*) was made. After each test, the orientation was changed to the opposite direction and also half of the test was in one orientation and other half in the opposite direction.

2.6.2 Photo sensitivity assay:

In photo sensitivity assay, a shorter A-B sensitivity apparatus having a total length of 40cm is used. And in all the orientations, the device is slightly and uniformly bend so that control and test sides didn't face each other and hence the light from the test side LED fall directly on the control side. If needed appropriate Light source (2.4.6.1) has to be used. The choice sides were fitted with two untreated cloth pieces each. Then batteries were placed in the battery holder and LED with wire and sleeve was connected to the battery holder. Thereafter glowing LED was placed inside the choice side hose adapter through its outside opening and kept at the center of the vertical cross-sectional circle with a distance of 2cm away from the cloth. And the connected battery holder was attached to the outside of the same hose adapter with rubber band. Then side cover (*section 2.4.6.2*) is placed over the test side, so that minimum/no composite light enter the test side. Required distraction reduction arrangement (*Section 2.4.5*) has made. As described (*section 2.6.1*), half of the test should be in one orientation and other half in opposite direction. In Figure 3, Multiple(six) short A-B sensitivity apparatus arrangement and also an assembled short A-B sensitivity apparatus with slight bend and LED light source are shown.

2.7 Result and Neutrality Assessment

2.7.1 Result Assessment:

2.7.1.1 Photo Sensitivity and preliminary evaluation test:
The result is recorded by counting the number of insects in the control and test sides after 15 minutes of insect introduction. preliminary evaluation test was replicated 6 times, while that of photo sensitivity test was 4 times.

2.7.1.2 Advanced test:
The repellent activity is recorded by considering the side where the insect reaches first. If at-least one insect reaches the control side first, the repellent activity is +100%. If at-least one insect reaches the test side first, the repellent activity is -100%. If the insect reaches both sides simultaneously or by a time difference of maximum 3 second, the repellent activity is 0. And the test was discarded after 2 min following the introduction of the insect. The test was replicated six times. The insect's delay in reaching the ends indicates its increased probability to escape from the path towards the target/ends. But the apparatus, force the insect to stay inside. The restrictions in time difference and test duration helps to avoid false negative/positive result if insect stays at a position (especially in test side) for long till the repellent/attractant gradient around the insect raises above its differentiation capacity. As the real-world scenario is open, the insects get the chance to escape from the repellent. The reliability of the result increases.

2.7.1.3. Absolute effective surface concentration:
The minimum surface concentration or absolute effective surface concentration in $\mu l/cm^2$ of the repellent that gives +100% repellent activity in all the 6 replicates was called absolute effective repellent dosage. And the minimum surface concentration or absolute effective surface concentration in $\mu l/cm^2$ of the repellent that gives -100% repellent activity in all the 6 replicates was called absolute effective attractant dosage.

The identification of exact absolute effective surface concentration was done by screening a range. Initially a range of 0.5µl (lower range) to 3µl (upper range) used. At first two replicates of Lower range (0.5µl) done. Two conditions arise, condition one was the positive result, which means that both replicates gave +100% repellent activity. In this scenario, another range with current lower range (0.5µl) as the new upper range was determined and screened again. Second condition was the negative result, where both or one of the experiment didn't gave +100% repellent activity. Then the upper range (here 3µl) was tested. If the result was negative, another range with the current upper range as lower range was determined (say 3-6µl) and screened again. But If the result was positive, the experiment was repeated with a range with the current upper range(3µl) as upper range and a value nearly in between the current upper range and lower range as the new lower range (say 1.5µl) and screened again. If the new lower range gives a positive answer, new range with the current

lower range (1.5µl) as the new upper range and the value just above lower range(0.75µl) of the previous test as the new lower range was screened. The above mentioned process was repeated till the possible minimum absolute concentration was reached. After reaching that point the six replicates of the concentration was tested. If it doesn't yield positive result in all the six tests, the next higher value was tested with six replicates.

2.7.2 Neutrality Assessment:

After setting up the A-B sensitivity apparatus as stated above, neutrality of the apparatus was assessed separately for chemical sensitivity and photo sensitivity test. In neutrality assessment test, both sides were kept in the same condition or in the control condition. Both the ends were loaded with two untreated cloth pieces each. *S.granarium* was added according to *section 2.5.* And the result was determined according to *section 2.7.1.2.*

The device is considered neutral when the device produces one result of 0% repellent activity and/or produce both -100% and +100% repellent activity in six replicates. No need of getting a mean of 0% in 6 attempts, because the desired mean repellent activity of 0% can be obtained in the first attempt or in 2 replicates or after tens of replicates. Because the required mean repellent activity of 0% is in the realm of probability and sometimes may require hundreds of replications. But the objective of device neutrality is proved, if the device gives 0% repellency and/or +100 and -100 repellencies in 6 replicates. Apparatus that fails this criterion were discarded.

2.8 Chemical sensitivity assay:

2.8.1 Preliminary evaluation tests:

Preliminary evaluation of botanical was done using Methanol-Water extract, Ethyl acetate extract and essential oil. Five ml of Methanol-Water extract or Ethyl acetate extract were added to a glass petridish having a dia-meter of 10cm containing one cloth piece (2.4.4). So, the cloth had a minimum surface concentration of 63.69µl /cm^2. In the case of essential oil used for preliminary evaluation, 1ml essential oil-acetone mixture was added to the glass petridish with one cloth piece. Therefore, the cloth had a minimum surface concentration of 0.3821µl essential oil per cm^2. While 5ml Methanol-Water mixture, 5ml Ethyl acetate and 1ml acetone were treated as control. Petridishes with Methanol-Water extract and Methanol-Water mixture were kept for twelve hours to remove the solvent. Ethyl acetate extract, Ethyl acetate, essential oil-acetone, acetone was kept for 3 hours to remove the solvents. All evaporation was conducted at 25ºC and standard pressure.

For the control side one piece of solvent treated cloth piece and another untreated cloth piece were kept over the half connector and for the test side one piece of extract or mixture treated cloth piece and another untreated cloth piece were kept over the half connector. In both the cases treated cloth faces the inner side of the main tube and untreated cloth faces the connector surface. Cloth's center coincides with the center of the face of the connector. And were inserted in to the main tube to a length of 2cm. Control side was primarily inserted and the test side. Then insect was introduced according to *section 2.5* and result was determined according to *section 2.7.1.1.*

2.8.2 Advanced analysis:

Here the test side and control side were loaded with two untreated cloth pieces each. Then to the test side required quantity of essential oil was applied directly to the central portion of the cloth piece through the opening at the other end of the connector using micro pipette. Then insect was introduced according to *section 2.5* and result was determined according to *section 2.7.* The Absolute effective surface concentration was determined according to the *section 2.7.1.3.*

2.9 Photo sensitivity assay

After setting up the A-B sensitivity apparatus as stated in the *section 2.6.2.*, insects were introduced according to *section 2.5* and result was determined according to *section 2.7.*

2.10 Statistical analysis

Documentation, charts and statistical analysis were done in Linux OS using LibreOffice (Version 5.4.1.2) (Writer, Calc and Math). Photographs were processed using Gimp (Version 2.8.20) and Inkscape (Version 0.92).

The essential oil yield was calculated by dividing the quantity of essential oil obtained in µl by weight of the raw material used in grams.

If Nc was the number of insects on the untreated area after the exposure interval and Nt was the number of insects on the treated area after the exposure interval. Percentage repellent activity ($R_{\%}$) was calculated using the formula (Nerio, 2009).

$$\textit{Percentage repellent activity}\left(R_{\%}\right)=\frac{N_c-N_t}{N_c+N_t}\times 100$$

A new index called Repellent Index(Ri) is used to represent and compare the strength of the repellents at different conditions of concentration and repellent activity. Repellent Index(Ri) is directly proportional to percentage repellent activity and inversely proportional to Surface volume concentration of repellent(Cr). In another words the strength of a repellent increases with repellent activity and decreases with the increase of Surface volume concentration of repellent(Cr) used. Ri has the unit, $cm^2/\mu l$. So,

$$Ri = \frac{R_{\%}}{Cr}$$

While another new index called Anti-Repellent Index(ARi) is the indicator of the strength of the different insects against repellents. Anti-Repellent Index(ARi) is directly proportional to the Cr and inversely proportional to the PR and mean weight(Wi) of the insect in grams. It has the unit $\mu l/gcm^2$. so,

$$ARi = \frac{Cr}{R_{\%} \times Wi}$$

Comparison of the sample variability relative to the mean was done by calculating the coefficient of variation expressed as a percentage (Zar, 2004).

In the photo-sensitivity test, $R_{\%}$ between darkness (minimum light situation), red, yellow, green, blue light and composite light were found. Composite light was considered as the control. The difference of mean and range, both positive and negative was used to describe the variation. Let $R_{\%C}$, $R_{\%D}$ and $R_{\%A}$ were the percentage repellent activity of the composite light, darkness and a (or any) color. The sensitivity between composite light and different colors were determined by calculating relative photo sensitivity. If $R_{\%C}$ (note : $R_{\%C} = -R_{\%D}$) is the percentage repellent activity of composite light versus darkness, here darkness is the considered as the control. Therefore, Relative Photo Sensitivity(RPS) is determined using the formula,

$$RPS = \frac{R_{\%A} - R_{\%C}}{R_{\%D}} \times 100$$

2.11 Analysis of biochemical composition.

Cardamom essential oil was analyzed using GC-FID with standards of major components. While essential oils of *Merremia vitifolia* and Peperomia Pelucida were analyzed using GC-MS. Probable compounds were identified using Main library(NIST) and FFNSC2 library.

RESULTS AND DISCUSSION

3.1 Essential oil yield

Only about a third of plants gave noticeable/useful yield of essential oil, they are *Cinnamomum malabatrum* (0.781µl/gm), *Peperomia pelucida* (0.578µl/gm), *Mikania scandens* (0.491µl/gm), *Afrocarpus gracilior* (0.167µl/gm), *Merremia vitifolia* (0.721µl/gm), *Pajanelia Longifolia* (0.8µl/gm) and *Tectona grandis* (0.175µl/gm).

3.2 Preliminary evaluation phase repellent activity

3.2.1 Methanol-Water extract:

All samples except *Corypha umbraculifera* (-1.18) and *Sarcotigma Kleini* (-16.39) showed repellent activity. While Cosmos sulphureus (94.44), *Merremia vitifolia* (79.68) showed the most repellent activity. Seeds of Tabernemontana Alternifolia showed higher repellent activiy than its leaf, but the leaf of hydnocarpus Laurifolia possessed more repellent activity than its seeds. While only Sarcotigma Kleini and Corypha umbraculifera were attractants, so excluded from Figure 4. Details of the result are shown in Table3 and Figure 4.

3.2.2 Ethyl acetate extract:

Like methanol-water extract, this extract of Sarcotigma Kleini (-18.61) acted as an attractant, while the percentage repellent activity of Averrhoa Bilimbi and Cuscuta Reflexia were below one. Therefore Averrhoa Bilimbi, Cuscuta Reflexia and Sarcotigma Kleini were excluded from Figure 5. Hydnocarpus Laurifolia (seed) showed the highest percentage repellent activity of 88.33. But the activity of the leafs of hydnocarpus Laurifolia showed lesser activity. The difference in activity between the Ethyl acetate extracts of the leaf and seed of Tabernemontana Alternifolia is lesser than that of its methanol-water extracts. While comparing the number of samples which had activity above 50%, ethyl acetate extract has only half of that of the methanol-water extract. Details of the result is shown in Table 3 and Figure 5.

3.2.3 Essential oil diluted with acetone:

Cinnamomum malabatrum (76.67) and Peperomia Pelucida (75.93) tops the list with higher repellent activity while the odd was Pajanelia Longifolia (6.75) with very low activity. Details of the result are shown in Table-4 and Figure-6.

Table 3: Repellent activity of Methanol-Water extract and Ethyl acetate extract.

S. no.	Plant	*Methanol-Water extract*			*Ethyl acetate extract*		
		Repellent Activity (%)	Coefficient of Variability (%)	Standard deviation	Repellent Activity (%)	Coefficient of Variability (%)	Standard deviation
1	*Cosmos sulphureus*	94.44	14.41	13.61	68.52	55.21	37.83
2	*Merremia vitifolia*	79.68	21.38	17.03	45.34	78.39	35.54
3	*Afrocarpus gracilior*	72.83	30.47	22.19	35.28	115.96	40.91
4	*Tectonia grandis*	72.59	45.56	33.07	3.44	975.84	33.6
5	*Pajanelia longifolia*	63.33	42.9	27.17	45.65	45.64	20.83
6	*Mikania scandens*	58.61	72.33	42.39	31.99	101.67	32.53
7	*Cassalia curviflora*	51.02	57.93	29.56	47.29	103.91	49.14
8	*Dendrophthoe falcata*	40.48	62.57	25.33	9.99	461.41	46.08
9	*Pithecellobium dulce*	39.62	154.09	61.04	19.74	80.37	15.86
10	*Tabernemontana alternifolia* (seed)	34.81	113.59	39.54	13.12	348.49	45.73
11	*Hydnocarpus laurifolia*(leaf)	32.41	70.81	22.95	35.38	59.73	21.14
12	*Peperomia pelucida*	24.26	215.42	52.26	14.64	188.21	27.56
13	*Dryneria quercifolia*	22.57	172.42	38.91	25.93	112.5	29.17
14	*Mimosa invisa*	22.22	188.23	41.83	1.67	2747.36	45.79
15	*hydnocarpus laurifolia* (seed)	17.58	245.17	43.1	88.33	14.62	12.91
16	*Cinnamomum malabatrum*	17.04	158.43	26.99	52.28	69.69	36.43
17	*Cuscuta reflexia*	15.56	279.21	43.43	0.71	5780.23	41.29
18	*Averrhoa bilimbi*	13.19	350.29	46.2	0.26	12008.5	31.77
19	*Tabernemontana alternifolia* (Leaf)	11.73	278.19	32.64	20.83	172.01	35.84
20	*Corypha umbraculifera*	-1.18	-3932.64	46.3	26.3	139.21	36.61
21	*Sarcotigma kleini*	-16.39	-121.16	19.86	-18.61	-82.16	15.29

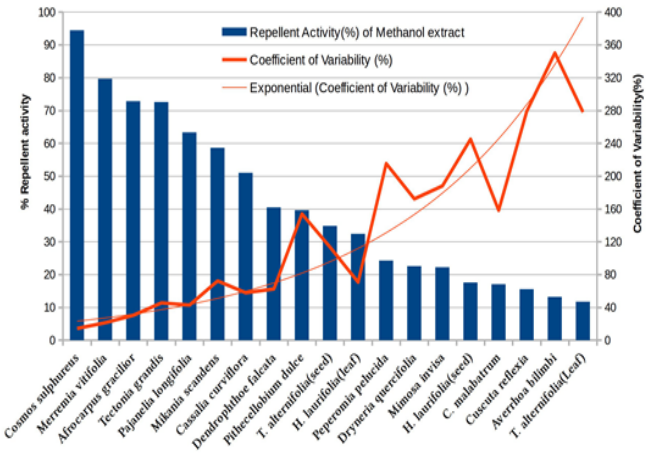


Figure 4: % Repellent activity and Coefficient of Variability of Methanol Extract

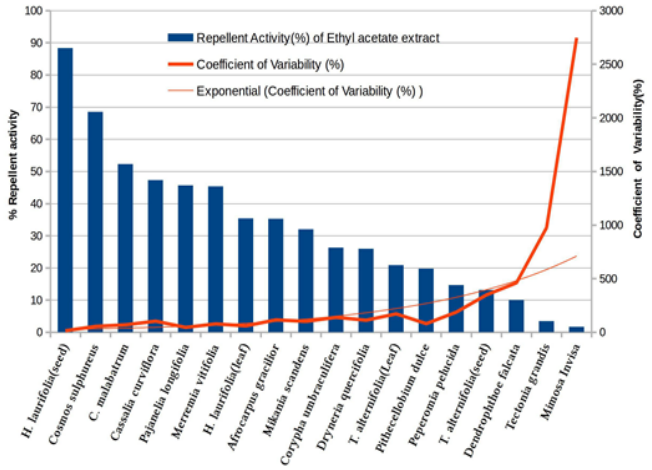


Figure 5: % Repellent activity and Coefficient of Variability of Ethyl Acetate Extract

Table 4: Repellent activity of essential oil diluted with acetone

Sl. no.	Plant	Repellent Activity (%)	Coefficient of Variability (%)	Standard deviation
1	*Cinnamomum malabatrum*	76.67	30.5	23.38
2	*Peperomia Pelucida*	75.93	10.38	7.88
3	*Tectonia Grandis*	70.34	41.27	29.03
4	*Mikania Scandens*	62.1	51.17	31.78
5	*Afrocarpus gracilior*	45.74	61.3	28.04
6	*Merremia vitifolia*	40.44	61.12	24.72
7	*Pajanelia Longifolia*	6.75	846	57.07

Table 5: Repellent activity of essential oils of *Elettaria cardamomum*(EC), *Merremia vitifolia*(MV) and Peperomia pelucida(PP).

Insect	mean Insect weight (mg)	Absolute Effective surface concentration (µl/cm²)			Repellent Index (cm²/µl)			Anti-Repellent Index (µl/gcm²)		
		EC	MV	PP	EC	MV	PP	EC	MV	PP
Henosepilachna vigintioctopunctata	8.3570	0.1975	0.1975	0.1975	506.45	506.45	506.45	0.2363	0.2363	0.2363
Pyrrhocoridae family	53.0	0.3949	0.1975	0.1975	253.23	506.45	506.45	0.0745	0.0373	0.0373
Sitophilus granarius	1.3519	0.7898	0.1975	0.3949	126.61	506.45	253.23	5.8421	1.4605	2.9210

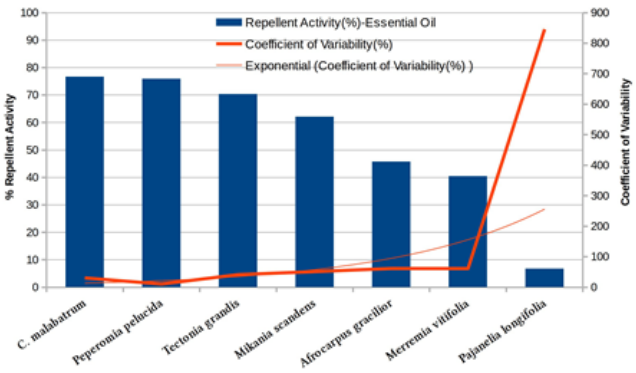


Figure 6: % Repellent activity and Coefficient of Variability of essential oil diluted with acetone.

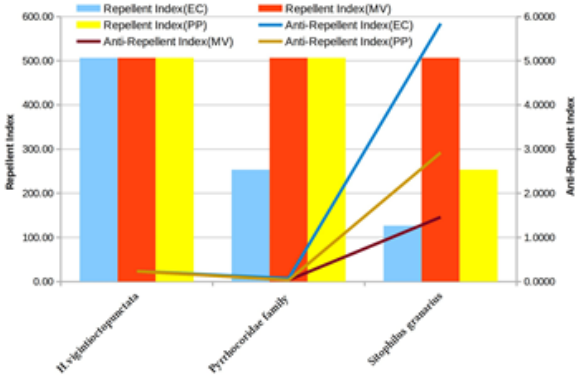


Figure 7: Repellent Index and Anti-Repellent Index of pure essential oils

3.2.4 Repellent Activity (%) and Coefficient of Variability (%):

It is also found that the Repellent Activity (%) and Coefficient of Variability (%) are inversely proportional. As the coefficient of variability (%) increases, the variation between the replication relative to the mean increases, so the results are more unpredictable, unreliable or erroneous with moderate number of replications. Therefore, the advanced stage test was focused on 100% repellent activity, so that the Coefficient of Variability (%) was nil.

3.3 Advanced phase repellent activity

Absolute Effective surface concentration (AESC) of *Merremia vitifolia* essential oil(0.1975µl/cm²) gave identical repellent activity on *Henosepilachna vigintioctopunctata*, Pyrrhocoridae family and *Sitophilus granarius*. *Henosepilachna vigintioctopunctata* have the same AESC of 0.1975µl/cm² for all essential oils. Essential oil of *Elettaria cardamomum* has the highest value of AESC against Pyrrhocoridae family and *Sitophilus granarius*. And only against *Sitophilus granarius*, the AESC of Peperomia Pelucida essential oil is

higher than that of *Merremia vitifolia*. Essential oil *Merremia vitifolia* has the strongest and that of *Elettaria cardamomum* has the weakest repellent Index. Sitophilus granaries (5.8421) has the strongest and Pyrrhocoridae family (0.0373) has the weakest Anti-Repellent Index, so *Sitophilus granarius* is the strongest insect against the strongest repellent. Details of the result are shown in Table 5 and Figure7.

Table 6: Repellent activity of certain insects with positive(+) and negative(-) range. Insects are S.granarius(SG), Pyrrhocoridae family(PF), H. vigintioctopunctata beetle(HB), H.vigintioctopunctata Grub(HG). Also its Relative Photo sensitivity.

Insect	*Parameters*	*Test Side*				
		Darkness	*Blue*	*Green*	*Yellow*	*Red*
SG	Percentage Repellent activity($R_{\%}$) Mean	22.50	26.07	8.33	-5.00	81.67
	+Range: Difference between maximum and mean $R_{\%}$	27.50	23.93	25.00	38.33	18.33
	-Range: Difference between minimum and mean $R_{\%}$	22.50	11.79	41.67	28.33	21.67
	Relative photo-sensitivity	0.00	215.87	137.04	77.78	462.96
PF	Percentage Repellent activity($R_{\%}$) Mean	57.50	83.33	21.67	-13.33	87.50
	+Range: Difference between maximum and mean $R_{\%}$	42.50	16.67	11.67	13.33	12.50
	-Range: Difference between minimum and mean $R_{\%}$	37.50	50.00	21.67	20.00	37.50
	Relative photo-sensitivity	0.00	244.93	137.68	76.81	252.17
HB	Percentage Repellent activity($R_{\%}$) Mean	100.00	-83.33	-100.00	-33.33	-25.00
	+Range: Difference between maximum and mean $R_{\%}$	0.00	16.67	0.00	33.33	25.00
	-Range: Difference between minimum and mean $R_{\%}$	0.00	16.67	0.00	33.33	41.67
	Relative photo-sensitivity	0.00	16.67	0.00	66.67	75.00
HG	Percentage Repellent activity($R_{\%}$) Mean	65.00	5.00	-100.00	-66.67	-33.33
	+Range: Difference between maximum and mean $R_{\%}$	35.00	28.33	0.00	33.33	33.33
	-Range: Difference between minimum and mean $R_{\%}$	31.67	38.33	0.00	33.33	33.33
	Relative photo-sensitivity	0.00	107.69	-53.85	-2.56	48.72

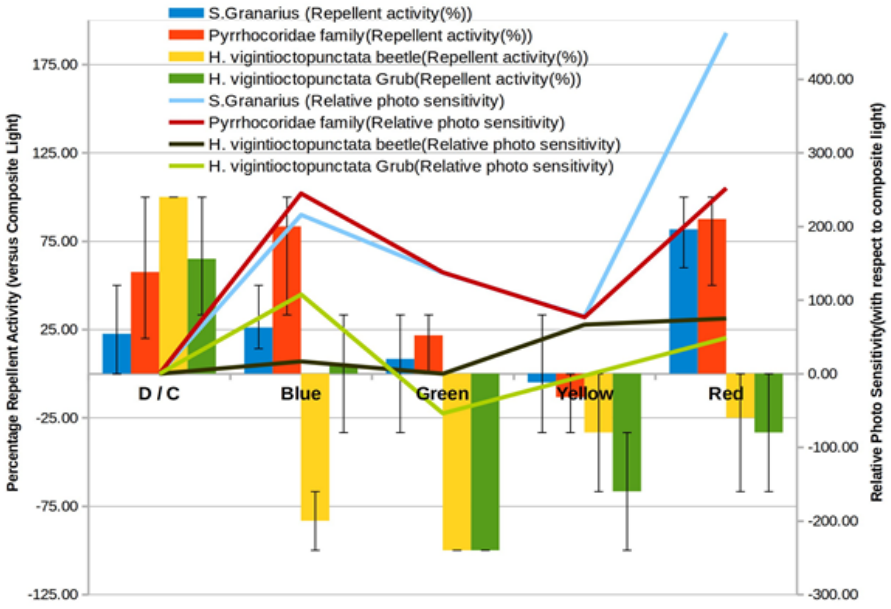


Figure 8: Percentage Photo Repellent activity of Insects towards different colors (with respect to darkness(D)). And Relative Photo Sensitivity towards different colors (with respect to composite light(C)).

3.4 Photo sensitivity

All insect species showed repellent activity against darkness or in other words all have affinity towards composite light. *S.granarius* was repelled by all the four colours, but more repelled by the colors with the most and least wavelength, that was red and blue. The pattern was more similar with the only other seed pest in the test, which was the Pyrrhocoridae family. Both have same sensitivity towards the middle of the spectrum, i.e, green and yellow. Except the grub, the color with the most repellent activity was red. *S.granarius* was the most sensitive insect in this case. Other than composite light, the seed feeding insects, *S.granarius* and Pyrrhocoridae family showed more affinity towards yellow light. Another adult in the test *H.vigintioctopunctata* beetle has 100 percent affinity towards composite and green light. The repellent effect of blue light was low. In other words, the sensitivity of *H.vigintioctopunctata* beetle towards blue and green light is identical to that of composite light. But after green the repellent activity increases towards red. But the *H.vigintioctopunctata* Grub was more attracted and had more sensitivity towards green light, the color of its food. So, all insect can distinguish colors and are sensitive at different levels. Seed feeding insects were more attracted towards yellow, while leaf eaters, grub and beetle of *H.vigintioctopunctata* are more attracted towards green. *H.vigintioctopunctata* beetle was less attracted and more sensitive towards the yellow color, the color of its grub. The details are shown in Table 6 and Figure 8.

3.5 Biochemical Composition

The major components of cardamom essential oil were α-Terpinyl acetate (53.1%), 1,8-Cineole (29.844%), Terpinen-4-ol (1.44%), Geraniol (1.218%), α-Pinene (0.814%), Linalool (0.706%), Linalyl acetate (0.46%), Sabinene (0.285%).

The probable compounds found in the essential oils of Peperomia Pelucida and *Merremia vitifolia* with their respective library were shown in the Table 7 and Table 8 respectively.

Table 7: Probable Compounds contained in Peperomia Pelucida essential oil.

RT (min)	*Probable Compounds Name*	*Area (%)*	*Library*
8.30	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	0.28	mainlib
8.30	3-Carene	0.28	mainlib
8.30	Carene <delta-3->	0.28	ffnsc2
8.30	Ocimene <(E)-, beta->	0.28	ffnsc2
8.30	Pinene <alpha->	0.28	ffnsc2
14.81	Bulnesene <alpha->	2.87	ffnsc2
14.81	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	2.87	mainlib
14.81	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	2.87	mainlib
14.81	Elemene <beta->	2.87	ffnsc2
14.81	Germacrene A	2.87	ffnsc2
15.33	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	3.88	mainlib
15.33	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	3.88	mainlib
15.33	Caryophyllene	3.88	mainlib
15.33	Caryophyllene <(E)->	3.88	ffnsc2
15.33	Caryophyllene <(Z)->	3.88	ffnsc2
16.36	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	4.50	mainlib
16.36	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aà,3bá,4á,7à,7aS*)]-	4.50	mainlib
16.36	Amorphene <gamma->	4.50	ffnsc2
16.36	Cubebene <beta->	4.50	ffnsc2
16.36	Germacrene D	4.50	ffnsc2
16.54	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	0.54	mainlib
16.54	Bicyclogermacrene	0.54	ffnsc2
16.54	Germacrene B	0.54	ffnsc2
16.54	ç-Elemene	0.54	mainlib
16.54	ç-Elemene	0.54	mainlib

17.38	1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	0.22	mainlib
17.38	2-Hydroxy-4-isopropyl-7-methoxytropone	0.22	mainlib
17.38	Benzene, 1,4-dimethoxy-2,3,5,6-tetramethyl-	0.22	mainlib
17.38	Benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy-	0.22	mainlib
17.38	Styrene <2,4,6-trimethyoxy->	0.22	ffnsc2
17.47	1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	0.23	mainlib
17.47	2-Hydroxy-4-isopropyl-7-methoxytropone	0.23	mainlib
17.47	Benzene, 1,4-dimethoxy-2,3,5,6-tetramethyl-	0.23	mainlib
17.47	Benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy-	0.23	mainlib
17.47	Styrene <2,4,6-trimethyoxy->	0.23	ffnsc2
18.16	1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	11.01	mainlib
18.16	2,5-Cyclohexadiene-1,4-dione, 3-methoxy-2-methyl-5-(1-methylethyl)-	11.01	mainlib
18.16	2-Hydroxy-4-isopropyl-7-methoxytropone	11.01	mainlib
18.16	3-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	11.01	mainlib
18.16	3-Oxabicyclo[3.3.0]octan-2-one, 7-neopentylidene-	11.01	mainlib
18.63	1-(3,3-Dimethyl-but-1-ynyl)-2,2,3,3-tetramethylcyclopropanecarboxylic acid	14.18	mainlib
18.63	5á,7áH,10à-Eudesm-11-en-1à-ol	14.18	mainlib
18.63	Dillapiole	14.18	ffnsc2
18.63	Patchouli alcohol	14.18	mainlib
18.63	[5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]hept-1-yl]-methanol	14.18	mainlib
18.90	2-Propenoic acid, 3-(3,4-dimethoxyphenyl)-, methyl ester	21.73	mainlib
18.90	Apiol	21.73	mainlib
18.90	Apiole	21.73	ffnsc2
18.90	Dillapiole	21.73	ffnsc2
18.90	Exalatacin	21.73	ffnsc2
19.00	2-Propenoic acid, 3-(3,4-dimethoxyphenyl)-, methyl ester	4.91	mainlib
19.00	Apiol	4.91	mainlib
19.00	Apiole	4.91	ffnsc2
19.00	Dillapiole	4.91	ffnsc2
19.00	Exalatacin	4.91	ffnsc2
19.09	2-Propenoic acid, 3-(3,4-dimethoxyphenyl)-, methyl ester	9.44	mainlib
19.09	Apiol	9.44	mainlib
19.09	Apiole	9.44	ffnsc2
19.09	Dillapiole	9.44	ffnsc2
19.09	Exalatacin	9.44	ffnsc2
19.26	1-(3,3-Dimethyl-but-1-ynyl)-2,2,3,3-tetramethylcyclopropanecarboxylic acid	17.09	mainlib
19.26	Apiol	17.09	mainlib
19.26	Apiole	17.09	ffnsc2
19.26	Dillapiole	17.09	ffnsc2
19.26	[5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]hept-1-yl]-methanol	17.09	mainlib
19.31	1-(3,3-Dimethyl-but-1-ynyl)-2,2,3,3-tetramethylcyclopropanecarboxylic acid	6.63	mainlib
19.31	Apiol	6.63	mainlib
19.31	Apiole	6.63	ffnsc2
19.31	Dillapiole	6.63	ffnsc2
19.31	Exalatacin	6.63	ffnsc2
19.62	1-(3,3-Dimethyl-but-1-ynyl)-2,2,3,3-tetramethylcyclopropanecarboxylic acid	2.48	mainlib
19.62	Apiol	2.48	mainlib
19.62	Apiole	2.48	ffnsc2
19.62	Dillapiole	2.48	ffnsc2
19.62	Exalatacin	2.48	ffnsc2

Table 8: Probable Compounds contained in *Merremia vitifolia* essential oil.

RT (min)	*Probable Compounds Name*	*Area (%)*	*Library*
7.60	(1H)Pyrrole-2-carbonitrile, 5-methyl-	0.59	mainlib
7.60	(1H)Pyrrole-3-carbonitrile, 2-methyl-	0.59	mainlib
7.60	4-Methylcoumarin-7,8-diyl dibenzoate	0.59	mainlib
7.60	Benzaldehyde	0.59	mainlib
7.60	Benzaldehyde	0.59	ffnsc2
7.94	(1H)Pyrrole-2-carbonitrile, 5-methyl-	0.29	mainlib
7.94	(1H)Pyrrole-3-carbonitrile, 2-methyl-	0.29	mainlib
7.94	Benzaldehyde	0.29	ffnsc2
7.94	Benzaldehyde	0.29	mainlib
7.94	Benzenemethanol, à-[1-(methylamino)ethyl]-, [S-(R*,S*)]-	0.29	mainlib
8.65	(1H)Pyrrole-2-carbonitrile, 5-methyl-	0.71	mainlib
8.65	(1H)Pyrrole-3-carbonitrile, 2-methyl-	0.71	mainlib
8.65	3-Pyridinecarbonitrile, 1,4-dihydro-	0.71	mainlib
8.65	Benzaldehyde	0.71	ffnsc2
8.65	Benzaldehyde	0.71	mainlib
8.73	(1H)Pyrrole-2-carbonitrile, 5-methyl-	0.76	mainlib
8.73	(1H)Pyrrole-3-carbonitrile, 2-methyl-	0.76	mainlib
8.73	3-Pyridinecarbonitrile, 1,4-dihydro-	0.76	mainlib
8.73	Benzaldehyde	0.76	ffnsc2
8.73	Benzaldehyde	0.76	mainlib
15.75	Bergamotene <beta-, trans->	0.13	ffnsc2
15.75	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	0.13	mainlib
15.75	Cedrene	0.13	mainlib
15.75	Di-epi-à-cedrene	0.13	mainlib
15.75	Longipinene <beta->	0.13	ffnsc2
16.30	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	0.21	mainlib
16.30	Caryophyllene	0.21	mainlib
16.30	Cedrene	0.21	mainlib
16.30	Germacrene D	0.21	ffnsc2
16.30	Longipinene <beta->	0.21	ffnsc2
16.71	Cedrene	2.01	mainlib
16.71	Cedrene <beta->	2.01	ffnsc2
16.71	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	2.01	mainlib
16.71	Germacrene D	2.01	ffnsc2
16.71	Sesquisabinene	2.01	ffnsc2
17.23	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1aà,4aá,7à,7aá,7bà)]-	27.73	mainlib
17.23	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-	27.73	mainlib
17.23	Longifolene-(V4)	27.73	mainlib
17.23	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aà)]-	27.73	mainlib
17.23	Patchoulene	27.73	mainlib
17.32	10s,11s-Himachala-3(12),4-diene	40.37	mainlib
17.32	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4à,4aá,7bà)]-	40.37	mainlib
17.32	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1aà,4aá,7à,7aá,7bà)]-	40.37	mainlib
17.32	1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1aà,3aà,7aá,7bà)]-	40.37	mainlib
17.32	Valerena-4,7(11)-diene	40.37	ffnsc2
17.42	.tau.-Cadinol	7.62	mainlib
17.42	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4à,4aá,7bà)]-	7.62	mainlib
17.42	Amorphene <delta->	7.62	ffnsc2
17.42	Cadinene <delta->	7.62	ffnsc2
17.42	Isoledene	7.62	mainlib
17.56	Cubenol	1.06	mainlib
17.56	Epiglobulol	1.06	mainlib
17.56	Globulol	1.06	mainlib

17.56	Ledol	1.06	mainlib
17.56	Sesquisabinene hydrate <trans->	1.06	ffnsc2
17.77	Cubenol	11.52	mainlib
17.77	Epiglobulol	11.52	mainlib
17.77	Globulol	11.52	mainlib
17.77	Ledol	11.52	mainlib
17.77	Sesquisabinene hydrate <trans->	11.52	ffnsc2
18.21	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	6.54	mainlib
18.21	Cubenol	6.54	mainlib
18.21	Epiglobulol	6.54	mainlib
18.21	Globulol	6.54	mainlib
18.21	Ledol	6.54	mainlib
18.89	Acorenol <alpha->	0.20	ffnsc2
18.89	Acorenol <alpha->	0.20	ffnsc2
18.89	Acorenol <beta->	0.20	ffnsc2
18.89	Cubenol	0.20	mainlib
18.89	Himachalol	0.20	ffnsc2
19.33	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1aà,4á,4aá,7à,7aá,7bà)]-	0.26	mainlib
19.33	3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	0.26	mainlib
19.33	Bisabolol <beta->	0.26	ffnsc2
19.33	Bisabolol <epi-beta->	0.26	ffnsc2
19.33	Sesquisabinene hydrate <trans->	0.26	ffnsc2

CONCLUSION

World is witnessing the insect resistance towards the most widely used synthetic insecticides, for example, the resistance report against neonicotinoid insecticide began to appear with in the third year of its introduction. The number of insect resistance reports began to increase even after the launch of new pesticides (Bass *et al.,* 2015). The newest technology of genetically modified crop plants also failed to completely eliminate the menace of synthetic pesticides (Coupe and Capel, 2016). Here also we need the service of natural enemy to delay the resistance of pests against genetically modified crops (Liu, 2014). So, in the long run genetically modified crops will face more problem than the problem of pesticide resistance. Reliance on hazardous pesticides is a short-term solution that undermines the rights to quality food and health for present and future generations (UN General Assembly, 2017). For example, Excessive mosquito population is one of the major indicator of unhygienic human habitats and the mosquito control mechanism fails, because major methodologies focus mainly on the direct eradication of mosquito(indicator), instead of correcting the real problem of biodiversity imbalance, unhygienic human habitats, etc. So, a positive result can be achieved only by changing the mindset from the lust for gouging the mother earth lest the huge ecological footprint of homo sapiens is reduced.

Being a natural product, botanical pesticides acts as a viable and safe substitution to a great extent. The repellent activity requires only less quantity of the essential oil per insect than that required for the same percentage of contact toxicity (Conti *et al,* 2011).

Considering certain methodologies with diffusion related errors, the percentage repellent activity wasn't directly proportional to the quantity of the repellent and also there were reduction in repellent activity with time. The A-B sensitivity apparatus and its methodologies, preserves the ideal and preliminary condition of pure diffusion but eliminate the errors due to diffusion of volatile compounds. The experiments showed that even one micro-liter of essential oil gave 100% repellent activity. The certain extracts in Preliminary evaluation test also gave respectable results of around 90%.

Pest's sensitivity to chemical and photo stimulus can be utilized to improve integrated pest management and is in the realm of further study. The method allows even enterprising farmer to test and then utilize the repellent activity of aqueous extract of weeds around their field. The result of the study emphasizes that if the purity and diversity of nature exists, effective nature friendly pest control strategies are possible.

The result of this study can pave way for many further researches in the realm of instrumentation, indices, more

insect species, potential/positive plants, phyto-chemicals, photo sensitivity with diverse wavelengths & luminosity, and pre-infestation effect of the repellent(s) &/attractant (s) application. Most plants selected for preliminary evaluation, doesn't had noticeable pest problem in its habitat. Therefore, the survival mechanism of plants with even negative test results also deserves further research. The A-B sensitivity apparatus with a modified IiT and/or IcT and/or Choice sides can effectively be used for analyzing preliminary repellent activity and anti-feedent activity of mosquito repellents.

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REFERENCES

Bass C, Denholm I, Williamson MS, and Nauen R (2015) The global status of insect resistance to neonicotinoid insecticides. *Pestic. Biochem. Physiol*. 121, 78–87.

Benelli G, Flamini G, Canale A, Molfetta I, Cioni PL, and Conti B (2012) Repellence of Hyptis suaveolens whole essential oil and major constituents against adults of the granary weevil *Sitophilus granarius*. *Bull. Insectology* 65, 177–183.

Conti B, Canale A, Cioni PL, Flamini G, and Rifici A (2011) Hyptis suaveolens and Hyptis spicigera (Lamiaceae) essential oils: Qualitative analysis, contact toxicity and repellent activity against *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae). *J. Pest Sci.* (2004). 84, 219–228.

Coupe RH and Capel PD (2016) Trends in pesticide use on soybean, corn and cotton since the introduction of major genetically modified crops in the United States. *Pest Manag. Sci*. 72, 1013–1022.

Drew DP, Rasmussen SK, Avato P, and Simonsen HT (2012) A comparison of headspace solid-phase microextraction and classic hydrodistillation for the identification of volatile constituents from thapsia spp. Provides insights into guaianolide biosynthesis in apiaceae. *Phytochem. Anal*. 23, 44–51.

Harborne JB (1998) Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis. Chapman & Hall.

Hori M (2003) Repellency of essential oils against the cigarette beetle, Lasioderma serricorne (Fabricius) (Coleoptera : Anobiidae). *Appl. Entomol. Zool*. 38, 467–473.

Iowa State University - Horticulture and Home Pest News. (2017) Rice Weevil and Granary Weevil. http://www.ipm.iastate.edu/ipm/iiin/ricewee.html. Date accessed: 31/03/2017.

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol*. 51, 45–66.

Liu X, Chen M, Collins HL, Onstad DW, Roush RT, Zhang Q, Earle ED and Shelton AM (2014) Natural enemies delay insect resistance to Bt crops. PLoS One 9. https://doi.org/10.1371/journal.pone.0090366

Lynch PA and Boots M (2016) Using evolution to generate sustainable malaria control with spatial repellents. Elife 5. https://doi.org/10.7554/eLife.15416

Matthews RW and Matthews JR (2010) The History and Scope of Insect Behavior. In Insect Behavior, pp. 1–44. https://doi.org/10.1007/s13398-014-0173-7.2

Nene YL (2003) Crop disease management practices in ancient medieval, and pre-modern India. Asian Agrihist. 7, 185–201.

Nerio LS, Olivero-Verbel J and Stashenko EE (2009) Repellent activity of essential oils from seven aromatic plants grown in Colombia against Sitophilus zeamais Motschulsky (Coleoptera). J. Stored Prod. Res. 45, 212–214.

Nerio LS, Olivero-Verbel J and Stashenko E (2010) Repellent activity of essential oils: A review. *Bioresour. Technol*. 101, 372–378.

Peter Atkins, Julio de Paula (2010) Atkins' physical chemistry, 9th edn. Oxford University Press. Oxford. pp. 773-774.

Piesik D and Wenda-Piesik A (2015) *Sitophilus granarius* responses to blends of five groups of cereal kernels and one group of plant volatiles. J. Stored Prod. Res. 63, 63–66.

Sallam Mohamed N (1999) INSECT DAMAGE: Damage on Post-harvest. In INPho- Post-Harvest Compendium, 38. www.fao.org/3/a-av013e.pdf(accessed 19 October 2017).

Seiber JN, Coats J, Duke SO and Gross AD (2014) Biopesticides: State of the Art and Future Opportunities. *J. Agric. Food Chem*. 62, 11613–11619.

Shimoda M and Honda K ichiro (2013) Insect reactions to light and its applications to pest management. *Appl. Entomol. Zool*. 48, 413–421.

Singh D (2014) Advances in plant biopesticides. https://doi.org/10.1007/978-81-322-2006-0.

Tang MJ, Shiraiwa M, Pöschl U, Cox RA and Kalberer M (2015) Compilation and evaluation of gas phase diffusion coefficients of reactive trace gases in the atmosphere: Volume 2. Diffusivities of organic compounds, pressure-normalised mean free paths, and average Knudsen numbers for gas uptake calculations. Atmos. Chem. Phys. 15, 5585–5598.

UN General Assembly (2017) An Agenda for Promotion and protection of all human rights, civil, political, economic, social and cultural rights, including the right to development, 27 February-24 March 2017, A/HRC/34/48, available at: http://undocs.org/en/A/HRC/34/ (accessed 19 October 2017)

Villaverde JJ, Sandín-España P, Sevilla-Morán B, López-Goti C and Alonso-Prados J L (2016). Biopesticides from natural products: Current development, legislative framework, and future trends. BioResources, 11(2), 5618–5640. https://doi.org/10.15376/biores.11.2.Villaverde

WHO (2017) Vector-borne diseases. http://www.who.int/mediacentre/factsheets/fs387/en/. (accessed: 31st Match 2017).

Zar JH (2004) Biostatistical Analysis, 4th edn. Pearson Education Inc. India. pp. 38-40.