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# Potential for Transmission of *Pseudomonas Aeruginosa* and Other Bacterial and Parasitic Infectious Agents by *Drosophila sp.* (Fruit Flies) as Mechanical Vectors

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**Abstract**
**Introduction:** *Drosophila melanogaster*, a fruit fly, has for more than 100 years played a pivotal role in scientific research, particularly in genetics and developmental biology. However, the *Drosophila* just like any other fly may act as a mechanical vector aiding in the transmision of parasitic and bacterial infectious agents. This fly has philia not only for fruits and moist food, but also for other moist environments including sinks, toilets, latrines, urinaries, sewages, soaps, sponges, towels, bathooms, mops, and plants. The fact that these flies alternate between these moist filthy environments and food suggests that they have the potential for transmiting pathogens such as *Entamoeba histolytica*, *Giardia lamblia,Trichuris trichiura, Ascaris lumbricoides, Salmonella tyhi, Shigella dysenteriae, Vibrio cholera, Escherichia coli, Campylobactor jejuni* ,and even, *Pseudomonas aeruginosa* from the moist filthy environments to food. Of particular concern is the possible relationship between *Pseudomonas aeruginosa* and *Drosophila*. *Pseudomonas aeruginosa* is an opportunistic pathogen, and one of the top three bacterial pathogens responsible for nosocomial infections.
**Hypotheses:** *Drosophila sp.* could be a mechanical vector for *Pseudomonas aeruginosa* and other bacterial and parasitic infectious agents. There could be a relationship between *Pseudomonas aeruginosa* and *Drosophila sp. Pseudomonas aeruginosa* thrives in moist environments, produces a fruity odor, and two types of soluble pigments: the fluorescent pigment pyoverdin and the blue pigment pyocyanin, just like a juicy fruit does hence attracting fruit flies to act as their mechanical vectors. This study is designed to investigate a possible role of *Drosophila sp.* as a mechanical vector of infectious agents.
**Methodology:** *D. melanogaster* samples were collected from bathrooms, toilets/latrines, kitchens and hospital wards using improvised **sterilized** *Drosophila* traps. These versatile traps (plastic containers; with a transparent base and colored lids) have a patented food grade attractant (a mixture of sterilized ripe banana paste and bread crumbs with small amounts of ethyl acetate added) that readily attracts several species of *Drosophila*. The baited flies were then analyzed for bacterial and parasitic infectious agents using culture and biochemical tests, and concentration techniques (e.g. formal ether), respectively. Determination of whether fruit flies may transmit various infectious agents or not, was achieved by computing their **proportions** for the 40 sampled areas analyzed. Determination of whether there is a statistically significant relationship between fruit fly attraction, bait type, and color, was achieved by comparing the geometric means of number of fruit flies for the various colors and bait type groups. T-test and ANOVA were used to compare difference in geometric means at significance levels of 5%.
**Results:** The most commonly isolated bacterial infectious agents were *P. aeruginosa* and *E. coli,* while parasitic infectious agents being *S. stercoralis. Candida albicans* the only fungal infectious agent was also isolated from *D. melanogaster.* The *P. aeruginosa* cultures attracted *D. melanogaster* while the different color types did not.
**Conclusion:** *D. melanogaster* is probably a mechanical vector for various bacterial, parasitic, and fungal pathogens. *P. aeruginosa* attracts *D. melanogaster* thus enhancing its transmission. To prevent the flies from breeding bushes should be cleared, dust bins emptied and cleaned regularly, and stagnating water drained around our residence and hospitals environment.
**Keywords**: Drosophila Melanogaster, Pseudomonas aeruginosa, Musca domestica, Strongyloides stercoralis, Rhamnolipid

## 1. INTRODUCTION.

### 1.1 The *Drosophila*

*Drosophila melanogaster*, is a fruit fly. For more than 100 years the flying insects have played a pivotal role in scientific research, particularly in genetics and developmental biology (Arnold, 2009). The name *D. melanogaster* in Greek implies the dark-bellied dew lover. These are flies of the order Diptera, and are commonly known as Fruit flies or vinegar flies. The flies are some of the most studied organisms in biological research because they are easy to take care of, breed fast, and lay many eggs. There are various types of *D. melanogaster*: the brown eyed with black body, cinnabar eyed, sepia eyed with ebony body, vermilion eyed, white eyed, and wild-type eyed with yellow body (figure 1). Similar to all insects *Drosophila* is covered in a

 

chitinous exoskeleton; has three main body segments; and has three pairs of segmented legs. They mature through complete metamorphosis (Miller, 2000). The adult common fruit fly is normally a yellow brown (tan) color, and is only about 3 mm in length and 2 mm in width. The shape of the common fruit fly's body is what one would normally imagine for a species of the order Diptera. It has a rounded head with large, red, compound eyes; three smaller simple eyes (ocelli), and short antennae (figure 1). Its mouth has developed for sopping up liquids. The female is slightly larger than the male. There are black stripes on the dorsal surface of its abdomen, which can be used to determine the sex of an individual. Males have a greater amount of black pigmentation concentrated at the posterior end of the abdomen. Like other flies, *D. melanogaster* has a single pair of wings that form from the middle segment of its thorax. Out of the last segment of its throax (which in other insects contains a second pair of wings) develops a set rudimentry wings that act as knobby balancing organs. These balancing organs are called halteres. Larvae are minute white maggots lacking legs and a defined head. The external morphology of the Drosophila fly is not different from that of *Musca domestica* (common house fly), except for the fact that the earlier posses the ocelli and the ptilinum a structure on the head for pushing the fly out of the puparium during developement. The mouth part is the proboscis, which comprises: the labrum, maxillae, and labium (Ferris, 1994). The fly belongs to Phylum: Arthropoda, Class: Insecta, Order: Diptera, Family: Drosophilidae, Genus: *Drosophila*, and Species: *melanogaster.*

**1.2. Geographical range, habitat, and behaviour**

*D. melanogaster* has been introduced to every continent of the world with one exception, Antarctica. On other continents its range is limited only by mountain ranges, deserts, and high lattitudes. The only aspects that limit the habitats *D. melangaster* can live in are temperature and availability of water.The development of this species' offspring is extremely dependent on temperature, and the adults cannot withstand the colder temperatures of high elevations or high altitude. Food supplies are also limited in these locations. Therefore, in colder climates *D. melanogaster* cannot survive. In temperate regions where human activities have introduced *D. melanogaster*, these flies seek shelter in colder winter months. Many times they can be found in fruit cellars, or other available man made structures with a large supply of food (Miller, 2000). The dew lovers will therefore inhabit most moist environments, ranging from food to bathrooms, hot-water sinks, canteens, hospitals, wet cloth, towels, latrines, soaps and even wounds (Colyer *et al.*, 1951). *D. melanogaster* are easily drawn towards the smell of any food source, and will mate almost indiscriminately with any individual of the opposite sex.They have hairs on their backs that are sensitive to air currents; their eyes are sensitive to slight differences in light intensity; and they will instinctively fly away when they sense a shadow or movement. They live primarily on plant material. The adults thrive on rotting plants, and fruits; while eggs are usually laid on unripened/slightly ripened fruit, so by the time the larva develop the fruit will have just started to rot, and they can use the fruit that the egg was laid on as their primary source of nutrition (Miller, 2000).

**1.3. Acquisition and transmission of infectious agents.**

Infectious agents refer to disease causing organisms (bacteria, parasites,viruses, and fungi) or stages of these organisms (eggs and cysts). Infectious agents can be categorised on the basis of how they are acquired (Talaro *et al.*, 1996). A disease is communicable when an infected host can transmit the infectious agent to another host. The transmission of the agent can be direct or indirect, and the ease with which the disease is transmitted varies considerably from one agent to the other. If the agent is highly transmissible, especially through direct contact, the disease is contagious.The transmitter of an infectious agent can be either openly infected or a carrier. Carriers are often humans or animals. Live animals that transmit infectious agents from one host to another or from a reservoir to a host, are called vectors. Most vectors are arthropods and include: mosquitoes, houseflies, Glossina, Chrysops, Culicoides, cockroaches, and fleas. Vectors are placed into two categories; mechanical and biological vectors. A biological vector is one that actively participates in a pathogen's life cycle, serving as a site in which it can multiply or complete its life cycle. On the other hand mechanical vectors are not necessary to the life cycle of an infectious agent, and merely transports it without being infected. The external body parts of these animals become contaminated when they come in physical contact with a source of pathogens. The agent is subsequently transferred to humans indirectly by an intermediate such as food or occasionally, by direct contact. Mechanical vectors have habits and adaptations that suit them to their role in disease transmission. Houseflies for example have mouthparts that are adapted to feeding on decaying garbage and faeces, which facilitate contamination of their feet and mouthparts. They also have hairs on their legs that help carry the infectious agents. In addition, while feeding they vomit and defecate the remains of their last meals (Schmidt *et al.*, 1989). Helminth eggs, protozoan cysts, and bacteria survive in the gut of the fly and thus can be widely distributed from the sites of their initial deposition.

**1.4. Hypotheses.**

*Drosophila sp. (*fruit fly) is a potential mechanical vector for *Pseudomonas aeruginosa,* and other bacterial and parasitic infectious agents. The fact that these flies have philia for moist habitats including filthy environment, and alternate between these areas and food, make them potentially capable of transmitting pathogens. These flies have adaptive features that enable them perform this role with ease just like the housefly. These include a

 

powerful sense of smell, well developed eyes for vision, and small hairs on the legs that enable infectious agents to be carried. *P. aeruginosa* could have an excellent and interesting relationship with the fruit flies that enable its transmission from the moist environment to solutions, sinks, plants, fruits, soaps, food, surgical instruments, towels, sponges, and even directly to undressed wounds. *P. aeruginosa (*a bacterial infectious agent found in moist areas) could have adapted various methods to increase its chances of transmission by the fruit fly. These include: pigment production, fruity odor production, and cohabitation with their proposed vectors in moist habitats.

$H_o$: Fruit flies do not transmit infectious agents.

$H_o$: There is no difference in the means of the number of flies attracted by the different colors.

$H_o$: There is no difference in the means of the number of flies attracted by the distilled water and rhamnolipid (2-aminoacetophenone).

**1.5. Objectives.**

The aim of this study is:

1. To find out if majority *of the Drosophila sp.* carries *P. aeruginosa,* and other bacterial and parasitic infectious agents.
2. To find out if more of the *Drosophila sp.* will be found trapped in traps with sweet smelling 2-aminoacetophenone compared to those with water.
3. To find out if the blue and green caped traps representing the colorful pigments produced by *P. aeruginosa* will contain more *Drosophila sp*. than the white caped.

**1.6. Justification of the study.**

*D. melanogaster* has same morphological features as those of *Musca domestica.* These flies also occupy similar habitats which include: garbage bins, food preparation areas, animal houses, slaughter houses, and areas where human waste has been deposited. In addition *D. melanogaster* inhabits ripe fruits, vegetables, sewer drains, mop pails, damp clothing, and other moist areas; which are all potential habitats for *P. aeruginosa.* During metabolism, *P. aeruginosa* produces a product known as the **Rhamnolipid;** this is a fatty acid ester with a characteristic fruity odor, similar to that found in fresh fruits like bananas or pears. The fruity odor in fruits is due to the presence of an ester known as ethyl acetate. **Ethyl acetate** (ethyl ethanoate, commonly abbreviated EtOAc or EA) is the organic compound with the formula $CH_3COOCH_2CH_3$. This colorless liquid has a characteristic sweet smell (similar to pear drops) like certain glues or nail polish removers, in which it is used. Ethyl acetate is the ester of ethanol and acetic acid; it is manufactured on a large scale for use as a solvent. Ethyl acetate is present in confectionery, perfumes, and fruits.The rhamnolipid is a viscous sticky oily yellowish brown liquid with a fruity odor. It shows solubility at aqueous pH≥4 with optimum solubility at pH 7–7.5 and freely soluble in ethyl acetate. Rhamnolipids are naturally occurring glycolipids produced commercially by *Ps. aeruginosa.* There are both mono-rhamnolipids and di-rhamnolipids. Containing only carbon, hydrogen, and oxygen, they are a combination of one or two rhamnose sugars and fatty acids. Rhamnolipids function as a natural surfactant, emulsifier, foaming agent, fungicide, antibiotic and anionic complexation agent. In a pure dry form, rhamnolipids are a white powder. In an aqueous solution they may range from clear to milky white or tan in color (Mohammad *et al.*, 2008). The surfactant is produced during anaerobiasis. When the products of *P. aeruginosa* are analyzed by gas chromatograpy-mass spectrometry, a number of volatile compounds are detected. Of significance here is the volatile compound; 2-aminoacetophenone (2-AA). It is this compound in the Rhamnolipid that is responsible for the grapelike odour (fruity smell) in *P. aeruginosa* cultures, and is used in its identification (Labows *et al.*, 1980). *P. aeruginosa* strains produce two types of soluble pigments, the fluorescent pigment pyoverdin and the blue pigment pyocyanin. The earlier is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium. Pyocyanin (from "pyocyaneus") refers to "blue pus", which is a characteristic of suppurative infections caused by *Pseudomonas aeruginosa*. A third but rare pigment that is red-brown (pyorubin), has also been observed to be produced by some strains of this organism (Todar, 2008). *D. melanogaster* perceives blue and green pigments, they are also attracted to the smell of fruits; hence there could be a relationship between the fly and *P. aeruginosa.*

## 2.0. LITERATURE REVIEW

*Musca domestica*, the domestic houseflies, are natural reservoirs and mechanical vectors of foodborne pathogens. Recent findings implicate flies as potential vectors for *E. coli* O157:H7 in beef or fruit products and *Salmonella enteritidis* in eggs. Scientific reports also implicate flies as reservoirs and vectors of enterohemorrhagic *E. coli* O157:H7 (EHEC-O157). These include epidemiological studies of the role of flies as vectors and reservoirs of EHEC-O157 in Obihiro City and Saga Prefecture, Japan, both sites of recent outbreaks of EHEC-O157. In the latter case, flies were found to harbor and proliferate EHEC-O157 (FAO, USA, 2001). The DNA pattern and vero-toxin were identical in the EHEC-O157 isolated from both patients and flies. Exclusion of flies from exposed food and utensils halted the Saga outbreak even though the excluded flies continued to test positive for EHEC-O157 (FAO, USA, 2001). In a study done in urban and rural Egyptian

 

districts, flies have been incriminated as mechanical vectors of many human diseases: diarrhoea, dysentery, typhoid, and conjuctivitis. Solid waste was found to increase fly density and hence the transmission and dissemination of fly born disease agents. Thirteen bacterial genera were identified from both waste samples and the associated flies; amongst them were *P. aeruginosa, S. aureus, and E. coli.* Some of the areas sampled included: market places, hospital garbage sites, industrial areas, and house hold garbage areas (Fallatah *et al.*, 2007). *Drosophila sp.* are a nuisance in homes, restaurants, fruit markets, canneries, especially when associated with decaying or rotting fruit and vegetables. Indoors, flies may be seen hovering around overripe fruit and vegetables, baked goods containing yeast, garbage cans and beverages such as fruit juices, cider, soft drinks, beer, wine and vinegar. Sometimes a rotten banana, potato, tomato, onion, melon, squash, pineapple or apple, dirty garbage receptacle, unclean sour mop or dishcloth, empty tomato catsup bottle, or drain water in refrigerators or iceboxes can yield a heavy population of these flies. Outdoors, they become numerous during summer and autumn where fruit and vegetables are harvested and then suddenly disappear when cold weather arrives. Some species are attracted to human and animal excrement, also feeding on fruits and uncooked foods, serving as a disease carrier (Lyon, 1991). In the Public-Health Pesticide Applicator Training Manual, *Drosophila sp.* have been classified as filth-breeding flies. Fruits, vegetables, beer, fermenting water from refrigerators, humidifiers, sink drains, sour mops and rags, and fermenting pet food are examples of their oviposition sites. Infestations are common in orchards, breweries, restaurants, supermarkets, canneries, hospitals and homes (University of Florida, 2009). Although these flies are generally harmless, some species like *D. replata* have been reported as potential means for mechanical transmission of pathogens when they breed in animal faeces (Mullen *et al.*, 2009).

A study done on *D. melanogaster* shows that they are susceptible to many of the infectious agents that cause disease in man. Hence they are considered the best models for studying human infectious diseases. Examples of pathogens used in the study include: *Ps. aeruginosa, Serratia marcescens, and Listeria monocytogenes.* These pathogens were found to cause different kinds of pathology in these flies, hence they can be considered potential carriers of the pathogens when infected (Dionne *et al.*, 2009). In a study where *D. melanogaster* was used as a model host for identification of *P. aeruginosa* mutants, it was found that the bacterium is so versatile that it is not only a major cause of opportunistic human infection but also virulent toward plants, insects, and the soil-dwelling nematode worm *Caenorhabditis elegans.* It was also found that *P. aeruginosa* PA01 kills *D. melanogaster,* and *P. aeruginosa* strain α1 is so lethal that the bacteria grow exponentially within the fly until and even after the death of the fly (D'Argenio *et al.,* 2001). Environmental studies have demonstrated a close association between *Vibrio cholerae* and many species of arthropods, and insects have previously been implicated as vectors of *V. cholerae* (Blow *et al.,* 2005). Researchers have also reported the susceptibility of the fruit fly, *D. melanogaster,* to oral *V. cholerae* infection through a process that exhibits many of the hallmarks of human disease. Furthermore, although ingestion of cholera toxin results in massive diarrhea in mammals, these researchers have found that ingestion of purified cholera toxin is not lethal to the fly. However, when co-ingested with a pathogenic strain of *V. cholerae* carrying a deletion of the cholera toxin genes, cholera toxin is lethal. These findings not only demonstrate the utility of *D. melanogaster* as an accurate, inexpensive model for elucidation of the host-pathogen interaction and identification of inhibitors of the action of cholera toxin; they also suggest that *V. cholerae* carries additional virulence factors that enable intoxication of an arthropod host. Based on these findings, the researchers suggest that the fly or a related arthropod may be a true host of *V. cholerae* in nature. Once ingested *V. cholerae* was able to multiply within the intestinal compartment and the bacterium was found to be concentrated in the midgut of the fly (Blow *et al.*, 2005). *Serratia marcescens* is an entomopathogenic bacterium that opportunistically infects a wide range of hosts, including humans. In a model of septic injury, if directly introduced into the body cavity of *Drosophila*, this pathogen is insensitive to the host's systemic immune response and kills flies in a day. In the study it was found that *S. marcescens* is resistant to the *Drosophila* immune deficiency. If ingested by *Drosophila*, bacteria cross the gut and penetrate the body cavity. During this passage, the bacteria can be observed within the cells of the intestinal epithelium. In such an oral infection model, the flies were found to succumb to the infection only after 6 days (Nehme *et al.*, 2007).

From the *Drosophila's*, life-cycle, it can be seen that it inhabits areas during its breeding cycle where bacteria, parasitic agents, fungi and viruses are likely to be located. These flies are, in general, harmless to man and do not bite but from recent studies there is evidence that they can carry pathogens. Outbreaks of infestation are often indicative of less than adequate hygiene or drainage problems. A recent study has implicated them in the spread of disease. Pathogenic *E. coli* O157:H7, as well as non- pathogenic strains, were found to grow exponentially in wounds on Golden Delicious apple fruit. The exponential growth occurred over a longer time period on fruit inoculated with a lower concentration of the bacterium than on fruit inoculated with a higher concentration. The bacterium reached the maximum population supported in the wounds regardless of the initial inoculum concentrations. Experiments on the transmission of *E. coli* by the flies, collected from a compost pile of decaying apples and peaches, were conducted with strain F-11775, a fluorescent transformant of non-

 

pathogenic *E. coli* ATCC 11775. The flies were easily contaminated externally and internally with *E. coli* F-11775 after contact with the bacterium source. The flies transmitted this bacterium to uncontaminated apple wounds, resulting in a high incidence of contaminated wounds. Populations of the bacterium in apple wounds increased signifcantly during the first 48 h after transmission (Anderson, 2009). Materials commonly infested by the flies include fruits, vegetables and fermenting liquids such as beer, cider, vinegar, and wine; some species are attracted to human and animal excrement. Because of their short life cycle of 8-10 days, they can exploit many temporarily available developmental sites. Dishwater and mop water full of food particles can accumulate on surfaces and/or in crevices and ferment, providing ideal fly breeding conditions. *P. aeruginosa* has frequently been found in aerators, traps of sinks, in baby whirlpool and hydrotherapy baths, in respiratory therapy equipment, and on showerheads. Swimming pools, hot tubs, contact lens solutions, cosmetics, illicit injectable drugs, artificial finger nails, inner soles of sneakers, anesthesia equipment, incubators, bathroom fixtures, utensils, and mops. Immunosuppressed individuals in most hospitals have been denied fruits and vegetables due to fear of gastrointestinal colonization by the organism. It is a part of normal flora of healthy individuals in the following areas: the gastrointestinal tract, the throat, norsal mucosa, and moist skin surfaces such as the axillae and the perinium. Rate of colonization increases in hospitalized individuals, with the highest being those hospitalized for lengthy periods of time; colonization sites for this patients include the lower respiratory tract (Murray *et al.*, 2003, Talaro, 2005, and Myrvik *et al.,* 1974). *P. aeruginosa* colonizes almost any moist surface on earth, just like the *D. melanogaster* hence increased chances of their interaction and hence transmission. The organism is normally introduced by visitors in hospital environment through flowers, plants and food brought to the patients (Topley *et al.*, 1998).

An annual increase in *Campylobacter* infection in England and Wales has been found to begin in May and to reach a maximum in early June. This increase occurs in all age groups and is seen in all geographic areas. Examination of risk factors that might explain this seasonal increase identified flies as a potential source of infection. The observed pattern of infection was hypothesized to reflect an annual epidemic caused by direct or indirect contamination of people by small quantities of infected material carried by flies that had been in contact with faeces. The possible seasonal drivers were examined, and only vector transmission by flies appeared to provide a convincing explanation for the observed seasonal trends. The seasonal increase in *Campylobacter* infections in May and June in England and Wales was hypothesized to reflect an annual epidemic caused by direct or indirect exposure of humans to contaminated material carried by several fly species that had been in contact with human, bird, or animal faeces or contaminated raw foods. Flies have been shown to carry *Campylobacter* and can infect both humans and animals. Intervention studies have demonstrated diarrheal disease reduction linked to control of flies, and deaths from diarrheal diseases have been linked to measurements of fly abundance. Disease transmission was hypothesized to occur through small quantities of contaminated material carried on the feet, proboscis, legs, and body hairs or from material regurgitated or defecated by flies. The variety, numbers, virulence and viability of organisms in the contaminated material differed, and some contamination included *Campylobacter* while others did not. Contamination of food by flies could occur at any stage of the food supply chain, but Campylobacter counts within the contaminated material on foods decreases over time; consequently, most infection result from contamination close to consumption. A number of synanthropic fly species were thought to be involved, including houseflies e.g., Musca spp., Fannia spp., blowflies e.g., Calliphora spp., Lucilia spp., and other dung-related flies e.g., Sarcophaga spp., and *Drosophila sp.*These flies have individual behavioral patterns, ecology, physiology, and temporal and geographic distributions that influence the likelihood of their being in kitchens, on human or animal faeces, and on food. Flies contaminated through fecal contact carry heterogeneous mixtures of organisms, including any pathogens that are present within the faeces, and may be able to cause a variety of human infections, including infection by different *Campylobacter* species and types. Gastrointestinal disease caused by flies is more likely to involve pathogens with a low infectious dose e.g., *Shigella, Campylobacter, Cryptosporidium, Giardia, Cyclospora, E. coli O157*), and some of these could have a seasonal component related to flies. Where high fly populations and poor hygiene conditions prevail, as in disasters or famines, or where pathogens can grow within fly-contaminated food, the potential exists for transmitting pathogens with a high infectious dose e.g., *Vibrio cholerae, Salmonella spp*. The access that flies have to human and animal faeces will influence the degree to which they are contaminated with different enteric pathogens. The hypothesis predicts that the *Campylobacter* infection rates will be higher in persons living close to animal production and lower in urban settings because fly numbers will be lower. Seasonal changes in *Campylobacter* incidence that are seen around the world may result from changes in fly populations and flies' access to human and animal faeces. Much emphasis on foodborne disease reduction has rightly been on kitchen hygiene, since the low infectious dose of *Campylobacter* makes cross-transmission from raw meats to ready-to-eat foods a substantial risk in domestic and catering environments. Fly transmission may be the most important source of infection in kitchen transmission routes, and establishments that sell ready-to-eat foods may be sources of *Campylobacter*, if effective fly control is not in operation. Flies may also be important in transmitting *Campylobacter* in poultry

 

flocks and between other agricultural animals (Gordon, 2005).

A study done on street vended fruit juice in Bellary city of India, indicated that the juice was contaminated by Coliforms; *Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogens and E. coli.* Most fruits contain bacterial counts up to 1.0 X $10^5$ $cm^2$ on their surfaces. Improper washing of fruits add these bacteria to the extracts leading to contamination. In addition, use of unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and *Drosophila sp.* and airborne dust were also implicated as sources of contamination. Foods and beverages prepared and sold by street vendors have contributed to transmission of cholera and enteric diseases in Latin America. Cholera transmission has been associated with consumption of street-vended beverages in Peru and Thailand (Reddy *et al.*, 2009). The *Erwinia* genus is a member of the Gram-negative Enterobacteriaceae family, and various species are phytopathogenic, causing soft rots of fleshy fruits, vegetables, and ornamentals. These pathogens have developed sustained plant-to-plant infection cycles, often via insect vectors such as bees and flies. *D. melanogaster* is a natural vector for *Erwinia carotovora atroseptica* and *Erwinia carotovora carotovora*, which cause potato blackleg disease. Bacterial species that infects *Drosophila* naturally are the species that are found in wild *Drosophila* environments. *D. melanogaster* feed on yeasts associated with decaying fruits and vegetables, which also provide food and a habitat for developing larvae. This environment is host to various bacterial species, and previous studies show that *D. melanogaster* serves as a vector for the phytopathogenic species of the bacterial genus *Erwinia*, that are associated with rotting plant matter. This study of the *Drosophila–Erwinia* interaction shows that insects are not passive carriers of these microorganisms, but, as observed, complex interactions can occur between the two partners. (Basset *et al.*, 2000).

Outbreaks of bacterial diseases associated with the consumption of fresh produce, such as lettuce, apple cider, unpasteurized apple juice, and alfalfa sprouts, have been reported with increasing frequency during the last decade. Although some produce-associated out-breaks may be due to cross-contamination from meat products, others more likely reflect direct contamination in the field with residues from faeces of wild or domestic animals. Direct contact with manure-contaminated soil or dust might be a source of preharvest contamination. Indirect sources of contamination could also be the trophic interactions between fruits and plant foragers like birds, mammals, and insects. The association between bacteria and fruit flies has usually been mentioned in relation to the source of attractants or in relation to symbiosis, which is important to the nutrition of the insects. Fruit flies have seldom been referred to as vectors of plant or human disease. The exceptions to this include the study of Cayol *et al*. (1989), which showed the potential capacity of the Mediterranean fruit fly (*Ceratitis capitata*) to transmit plant disease, and more recently, the study of Janisiewicz *et al*. (2000), which showed the possible involvement of the vinegar fly (*D. melanogaster*) in the transmission of pathogenic bacteria to postharvest wounded apples.The ability of *C. Capitata* to serve as a vector for food-borne pathogens has not been reported previously, although several features of this insect suggest its potential as a vector. *C. capitata* is a generalist cosmopolitan pest that infests more than 200 species of commercial and wild fruits. Like most flies, fruit flies must feed on protein in order to develop eggs. The protein sources for the *C. capitata* include rotting fruits and fecal material. Most fruit flies locate protein food sources through attraction to ammonium-releasing substances.This biological phenomenon was used by Pin ero *et al*. (2002). To develop an inexpensive attractant made of human urine and chicken faeces for monitoring of fruit flies by resource-poor fruit farmers. After reaching sexual maturity and copulating, female flies lay eggs in fruit by puncturing the skin of the fruit with their ovipositors about 1 to 2 mm deep and injecting batches of eggs into the wounds. First-instar larvae hatch from the eggs 2 to 3 days later, and 2 more instars feed on the fruit tissue. The attraction of the *C. capitata* to a variety of fecal material and its foraging on this material as a nitrogen source, in conjunction with its egg-laying activity in a variety of fruits and its extensive prevalence in numerous agricultural regions in the world, suggests that this fly potentially is a vector for faeces-borne pathogens. In this study the capacity of this fly to transmit *Escherichia coli* from contaminated fecal material to intact apples was investigated. Addition of green fluorescent protein (GFP)-tagged *E. coli* to a *C. capitata* feeding solution resulted in a dose-dependent increase in the fly's bacterial load. Flies exposed to fecal material enriched with GFP-tagged *E. coli* were similarly contaminated and were capable of transmitting *E. coli* to intact apples in a cage model system. Washing contaminated apples with tap water did not eliminate the *E. coli*. Flies inoculated with *E. coli* harbored the bacteria for up to 7 days following contamination. Fluorescence microscopy demonstrated that the majority of fluorescent bacteria were confined along the pseudotrachea in the labelum edge of the fly proboscis. Wild flies captured at various geographic locations were found to carry coliforms, and in some cases presumptive identification of *E. coli* was made. The vinegar fly (*D. melanogaster*) has also been implicated in the transmission of pathogenic *E. coli* to wounded apple tissues under laboratory conditions. These findings support the hypothesis that the *C. Capitata* is a potential vector of human pathogens to fruits.The similarity by which *D. melanogaster* comes in contact with food and faeces in its life cycle, suggests that it could also be a vector for human pathogens (Sela *et al*., 2005).

 

## 3.0. MATERIALS AND METHODS.

### 3.1. Study sites

The study was conducted in Embul-bul area in Ngong, and Kenyatta National Hospital (KNH) wards and kitchen area. Embul-bul area is a center containing shops, small kiosks, restaurants, residential houses, schools, and a health center. Most of the people in this area run businesses, ranging from: shops, kiosks, butcheries, rental houses and groceries. Some individuals in this area are farmers. The community shares most of the facilities including bathrooms and toilets, creating a good environment for spread of diseases in case of an epidemic. The soil in Embul-bul area is black cotton soil which drains poorly and so retains much of the water after the rains. This forms moist environments, and hence is a good habitat for breeding of Fruit flies, and explains the large population of these flies in the area.

### 3.2. The *Drosophila* Trap and Sample collection

In the study, *D. melanogaster* samples were collected from their moist habitat (bathrooms, toilets/latrines, kitchens, and hospital wards using improvised **sterilized** *Drosophila* bait traps (figure 2). The bait used for attraction in the traps, was then analyzed for bacteria using culture biochemical tests and for parasitic infectious agents using concentration techniques (Ogbu, 2007). The assumption here was that the flies would contaminate the bait by deficating on it and also shed off the infectious agents present on their body surfaces on to the bait. These versatile traps (plastic containers; with a transparent base and colored lids) have a patented food grade attractant (a mixture of sterilized ripe banana paste and bread crumbs with small amounts of ethyl acetate added) that readily attracts several species of *Drosophila*. Traps for bacteriological analysis contained additional nutrient broth to help in the culture of bacteria. Trap efficacy was compared by use of blue, yellow-green, orange-colored, and colorless (control) lids (1 set). The Vector fruit Fly Trap is engineered with a ten (2.5 mm diameter) hole venting lid to optimize *Drosophila* exposure to the odor emitted from the bait attractant. The holes are small, designed not to allow intruders in the trap, and if by chance intruders were found then the trap was discarded. The catch was analyzed, counted, and compared for each of the traps. The bait attractant in the traps was also replaced with water to act as a control and *P. aeruginosa* broth cultures and the results compared with those of the actual bait after laying the traps. All traps were set out in the study sites for equal amount of time (1 hour) before being collected, sealed and transported to the laboratory for study. The Rhamnolipid surfactant which contains the sweet smelling compound; 2-aminoacetophenone (2-AA), was produced by the *P. aeruginosa* in the broth cultures. This was achieved by the Rhamnosyltransferase 1 encoding gene: ***rhlAB,*** found in *P. aeruginosa*. Because the biosynthetic pathway for 2-AA branches from the tryptophan catabolic pathway, tryptophan was incorporated into the broth in 10ml concentrations to increase the production of 2-AA (Cox *et al*., 1979). During laying of the traps, any attractants e.g. dust bins, were removed from the sample collection areas to prevent distraction. The bait traps were also set on wooden stands to avoid environmental contamination.

An arbitrary 40 sample areas were analyzed: 10 from the hospital environment, and 30 from Embul-bul area. Sampling was done randomly using a total of 800 traps, with 30 out of 40 habitats being sampled in Embul-bul area. A sample consisted of 20 traps (5 sets): 2 sets holding the original bait, 2 sets contained the rhamnolipid surfactant (for non-hospital environments) and 1 set had water to act as a control. For the rhamnolipid and the food attractant traps; 1 set was for bacteriological analysis and the other for parasitological analysis. Traps with *P. aeruginosa* cultures were not used in the hospital environments because of ethical considerations. The sampling process was done twice a week, that is, on Mondays and Wednesdays, for a period of 5 months. Some *D. melanogaster* were also collected in small transparent sterile bottles for direct analysis so as to act as a control. The traps were transported immediately after the sampling process to the Department of Medical Microbiology, University of Nairobi where culture, laboratory tests and identification of bacterial and parasitic infectious agents was done.

### 3.3. Trap analysis

Four food samples and 1 direct sample from each of the sampling areas were analyzed for presence of infectious agents; giving a **sample size of 160,** the direct sample being used as controls. The average number of flies trapped by the different colored traps of different bait types was analyzed giving a **sample size of 160**. At the lab, the outer surfaces of the traps were wiped using ethyl alcohol, and the number of *D. melanogaster* in each of the traps counted and recorded.

#### a) Parasitological sets

The parasitological sets were analyzed on the same day for parasitic infectious agents and the results recorded. The parasitic infectious agents (ova and cysts) were concentrated from the bait attractants by formal-ether concentration technique, and the agents observed under a microscope after staining using iodine (Ogbu, 2007). For the concentration method, about 1g of the bait was emulsified in 7ml of formal saline and sieved through 4-layered gauze. Three milliliters of ether was then added to the filtrate in a centrifuge tube, shaken and centrifuged at 2000rpm for 2 minutes. The supernatant was then discarded and the deposit examined under a

 

microscope using ten and fourty times magnification after staining it with iodine. The *D. melanogaster* direct samples were analyzed on the same day of collection for parasitic infectious agents by concentration method after their collection in formal saline (Figure.19). The parasitic infectious agents that were looked for include: *Giardia lamblia and Entamoeba histolytica cysts, Trichuris trichiura,* and *Ascaris lumbricoides* ova, and any other pararasitic infectious agents of medical importance.

**b) Bacteriological sets**

From the bacteriological sets, cultures were done on selective media to help isolate the bacteria of interest from the mixture. Culture on specific media of choice was done as follows: on, Cetrimide media (CM), and incubation done at $37^0$C; Salmonella-Shigella agar (SS), and incubation done at $37^0$C; Thiosulphate-Citrate-Bile salt-Sucrose (TCBS), and incubation done at $37^0$C; Skirrow's medium (SK), in 5% $CO_2$ jars, and at $42^0$C; MacConkey's agar (Mac), and incubation done at $37^0$C and in addition Blood agar and incubation done in 5% $CO_2$ jars at $37^0$C. The bacteria in the remnant bait of the bacteriological sets were then inoculated into peptone water and incubated at $37^0$C till the following day. Peptone water was also added to the bacteriological analysis direct sample bottle, shaken and cultures done on selective media, the remaining potion was incubated at 37˚C. The following day, subcultures were done from the peptone water to all the selective media. On this day gram staining and biochemical analysis was done on the growth colonies on the cultures plates, while for the subcultures was done on the third day in order to identify the cultured bacteria. *P. aeruginosa* culture bait traps were not analyzed for the presence of organisms. Culture, biochemical tests, and the identity results were then recorded awaiting statistical analysis. The infectious agents were only identified but not quantified in both cases. For gram staining, a colony was emulsified using normal saline and a smear made on a glass slide (2.5cm in diameter), after air drying it was fixed by passing through a Bunsen flame three times (the slide should just be hot enough to be tested at the back of the hand comfortably). After cooling, the smear was covered with methyl violet for about 30 seconds.The stain was then drained off, and the slide washed gently with tap water. The smear was then covered with Gram's iodine for 30 seconds afterwhich it was washed off with water, and holding the slide at an angle, acetone-alcohol added drop by drop for about 5 – 10 seconds. Immediately, this was rinsed in water and stained with neutral red for 1 minute before being washed in water, blot dried on clean filter paper, and then examined under a microscope using oil immersion. Gram – positive organisms appear dark violet while gram – negative organisms are pink (Achola *et al*., 2001). The cultures on various media were analyzed by use of biochemical tests. Budding gram positive organisms were inoculated in plasma and incubation done at $37^0$C for 2 – 3 hrs (GTT test), the plasma was then observed using ten times magnification.

### 3.4. Data analysis

Data for hospital environment and non-hospital environment samples were handled and analyzed together.

a) Infectious agents:

$H_0$: Fruit flies do not transmit infectious agents.

$H_a$: Fruit flies transmit infectious agents.

Determination of whether fruit flies may transmit various infectious agents or not, was achieved by computing their **proportions** for the 160 samples analyzed.

b) Color:

$H_0$: There is no difference in the means of the number of flies attracted by the different colors.

$\mu_{CL} = \mu_{CR}$ or $\mu_{CL} - \mu_{CR} = 0$. Where CL—Is Colorless and CR—Is Colored.

$H_a$: There is a difference in the means of the number of flies attracted by the different colors.

$\mu_{CL} \neq \mu_{CR.}$

c) Rhamnolipid:

$H_0$: There is no difference in the means of the number of the flies attracted by the distilled water, food and the rhamnolipid (2-aminoacetophenone).

$\mu_W = \mu_R$ or $\mu_W - \mu_R = 0$.

$H_a$: There is a difference in the means of the number of flies attracted by the distilled water, food and rhamnolipid (2-aminoacetophenone).

$\mu_W \neq \mu_R$.

Determination of whether there is a statistically significant relationship between fruit fly attraction, bait type, and color, was achieved by comparing the geometric means of number of fruit flies for the various colors and bait type groups. For normally distributed level of significance of 5% data, t-test and ANOVA were used to compare difference in means.

## 4.0. RESULTS

### Parasitic, Baterial and Fungal infectious agents and *D. melanogaster*

A total of 160 *D. melanogaster* food bait samples and 40 direct samples were processed. The 160 *Pseudomonas* culture baits were not considered in this section since the objective was to establish mechanical transmission of infectious agents to food. Each of the *D. melanogaster* food bait samples had infectious parasitic, bacterial or

 

fungal agents isolated them. The infectious parasitic agents isolated consisted of *Trichuris trichura* ova, *Ascaris lumbricoides* ova, *Heterophyes heterophyes* ova, and *Strongyloides stercoralis* rhabditiform larvae. The infectious bacterial agents isolated were *Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae, Salmonella typhi, Campylobacter jejuni, Streptococcus pyogenes, Proteus vulgaris, Proteus mirabilis, Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus agalatiae, Staphylococcus aureus, Serratia marcescens, Salmonella paratyphi, Streptococcus pneumonia, Klebsiella oxytoca, Klebsiella pneumonia, Vibrio parahaemolyticus, Enterococcus faecalis, Citrobacter freudii, Shigella sonnei, and Shigella flexneri*. The only infectious fungal agent isolated was *Candida albicans* from some of the *D. melanogaster* bait samples. The proportions of the *D. Melanogaster* food bait samples containing these infectious agents were computed, while the direct samples were used as a control to confirm that the infectious agents in the baits were transmitted by the flies. The most commoly isolated infectious parasitic agents was *S. stercoralis.* This was found in 37.5% of the *D. melanogaster* bait samples. The other commoly isolated parasitic agents were *E. coli* (15%), *T. trichura* (10%) and *A. lumbricoides* (7.5%). See figure 3. Some of the infectious bacterial and fungal agents isolated were *P. aeruginosa* (97.5%), *E. coli* (75%), *K. oxytoca* (60%), *E. faecalis* (45%), *C. freudii* (42.5%), *S. pneumonia* (35%) and *S. flexneri* (35%). See figure 4. Figures 5 – 8 show photograps of the infectious agents of medical importance and other organisms isolated from *D. Melanogaster* bait samples.

**Relationship between type of bait and the numbers of *D. melanogaster* attracted.**

The mean number of *D. melanogaster* flies attracted by food baits was 7.187 while the mean number of *D. melanogaster* flies attracted by *P. aeruginosa* culture baits was 1.032. This difference was significant student's t-test (p-value < 0.05). Water baits attracted a mean of 0.00 flies. See figure 9. A total of 160 food baits and a total of 160 *P. aeruginosa* culture baits were used.

**Relationship between color types and the numbers of *D. melanogaster* flies attracted.**

The mean number of flies attracted by the various color types was as follows: blue food traps 1.147(95% CI) compared to *P. aeruginosa* culture bait traps 1.09, yellow green food traps 1.155(95% CI) compared to *P. aeruginosa* culture bait traps 1.102, orange food traps 1.15(95% CI) compared to *P. aeruginosa* culture bait traps 1.091, and colorless food traps 1.157(95% CI) compared to *P. aeruginosa* culture bait traps 1.096. *D. melanogaster* showed no particular preference for any of the colors. See figure 10. The P values for the food traps and *P. aeruginosa* culture bait traps were 0.283 and 0.380 respectively.

## 5.0: DISCUSSION

Transmission of pathogens by synanthropic insects is predominantly mechanical. In adult flies it occurs via mechanical dislodgement from the exoskeleton, fecal deposition, and regurgitation. Flies can carry human pathogens on the sponging mouthparts, on body and leg hairs (i.e., setae), or on the sticky pads of the feet (i.e., tarsi). Fine hairs on the pads of a fly's feet are coated with a sticky substance which improves the fly's ability to adhere while resting or climbing on no horizontal surfaces. This substance also enhances the adhesion of particles, i.e., viruses, bacteria, and protozoan cysts, to fly legs, which then can be directly transported to the next visited surface and dislodged. Small particles readily adhere to a fly's exterior surfaces due to their electrostatic charge. Fly exoskeletons have certain electrostatic charges and any particle with a different charge or a neutral charge will adhere to the fly surface (Hadi, 2011). Studies on *Musca domestica* have isolated various pathogens on their body surfaces. Vazirianzadeh *et al.* (2008) isolated *Escherichia coli, Staphylococci aureus*, *Pseudomonas spp*., and *Proteus spp*. Rochon (2003) also found out in her study in Canada that 78% of the adult *M. domestica* carry *E. coli* bacteria on their body surfaces. Ugbogu *et al.* (2006) isolated *Salmonella* and *Shigella* species from *Musca domestica* from 61.7% and 100% captured using a sweep net in Nigeria. The flies pooled from the refuse dump sites had a high load of these organisms. This previous study on *M. domestica* is similar to my study on *D. melanogaster* since proportions of bacteria from pooled samples of flies were used rather than from individual flies.

This current study suggests that *D. melanogaster* is a potential mechanical vector of infectious diseases agents. To my knowledge, this is the first time that a study examining the role of *D. melanogaster* as a mechanical vector has been conducted. A previous study looking at the potential role of other arthropods in mechanical transmission of infectious agents has compared the presence of these infectious agents on the body surfaces of the arthropods with the presence of these infectious agents on the body surface of *Musca domestica* the prototype mechanical vector Lamiaa *et al.* (2007). Lamiaa *et al.* (2007) compared the transmitted bacteria between houseflies and American cockroach, *Periplaneta americana*. They isolated *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus spp*., *Salmonella spp*., *Shigella spp.*, *Proteus vulgaris*, *Proteus spp, Serratia spp.*, *Klebsiella spp*., *Enterobacter spp*. and *Escherichia coli* from the external surface of both insects, and concluded that *Periplaneta americana* may be a potential mechanical vector of these infectious agents. The hypothesis that *D. melanogaster* could be a mechanical vector is further strengthened by the fact that *Drosophilidae* and *Musca domestica,* have common habitat, and have similarities in their body morphology.

However, there are studies that have examined the presence of pathogens beyond the body surface of

 

*Musca domestica.* These include those of Ostrolenk *et al.* (1942), where they were able to demonstrate that *M. domestica* flies are an extremely potent source of pollution organisms, particularly in those food plants where little attention is paid to sanitation and where the food is prepared for the consumer without a final treatment to destroy these organisms. They were able to demonstrate that *Salmonella enteritidis* may be transmitted with ease and rapidity through several populations of flies, and that all surfaces with which the infected fly came in contact with became potential sources of reinfection for other insects. Their study showed that not only do the flies carry the organisms on their outside surfaces, but also in their digestive tracts, since the infected, macerated insect contained large numbers of the test organism while the organism could not be isolated from the chemically treated, washed flies. Nazni *et al.*(2005), isolated *Bacillus* sp., *Coccobacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Enterobacter* sp., *Proteus* sp., *Escherichia* sp., *Klebsiella* sp. and yeast cells from feaces, vomitus, external surfaces and internal organs of house fly. In their study they examined flies from various breeding sites such as food courts, dumping ground, food processing areas and poultry farm in Peninsular Malaysia. The flies were baited with 10% sugar solution on a glass slide in the field. They also dissected adult flies and examined the gut contents.

In the present study, the most frequently trapped bacterial pathogens were *P. aeruginosa* and *E.coli* in 97.5% and 75% of the *D. melanogaster* baits, respectively. This is similar to findings of a hospital-based study by Kassiri *et al.* (2012) for *Musca domestica*, where the most frequently isolated bacterial pathogens were *Pseudonomans sp* (100%) and *E. coli* (80%). It can be concluded that just as *Musca domestica* is capable of mechanically transmitting *P. aeruginosa* pathogens, the same may be transmitted by *D. melanogaster* thus being an important factor in nosocomial infections. Isolation of *Streptococcus pneumonia* from *Drosophila melanogaster* has no public health implications since this bacterium is known to be transmitted via contamination of the respiratory tract by droplets or other materials containing respiratory secretions and not through the faeco- oral route. Other past studies that support this current study on mechanical transmission of bacterial infectious agents by *D. melanogaster* include those of Butler *et al.* (2010), who were able to isolate among others *Staphylococcus saprophyticus, E. coli, and Shigella dysenteriae* by PCR from *M. domestica* collected near the rear entrances and dumpsters of 4 restaurants in north central Florida. Habeeb *et al.* (2012), in their study to investigate mechanical transmission of bacterial via truefly species sampled out five species namely, *Musca domestica* (Muscidae), *Calliphora vomitoria, Calliphora vicina, Lucilia cuprina* (Calliphoridae), and *Sarcophaga haemorrhoidalis* from a market in Iraq. From the study it was found that *E. coli* was dominant among the flies, the other bacteria that were isolated included *Klebsiella* sp., *Staphylococcus, Enterococcus, Streptococcus, Proteus mirabilis,* and *Pseudomonas aeruginosa.*

Nwangwu *et al* (2013) used an approach similar to the one that I used in my study to capture *M. domestica* samples. To investigate the parasites associated with wild-caught houseflies in Awka metropolis, Anambra State, southeastern Nigeria, locally designed fly traps were used to collect flies. Parasites isolated from the flies were *Entamoeba histolytica cysts, Hookworm ova, Ascaris lumbricoides ova, and Trichuris trichiura ova.* These results agree with those of my study conducted for *D. melanogaster.* Gehad *et al.* (2011) in their study carried out in Egypt to investigate the role of cockroaches and flies in mechanical transmission of medical important parasites, isolated cysts of *Entomoeba histolytica*, oocysts of *C. parvum, C. cayetenensis* and *Isospora belli,* cysts of *Balantidium coli*, ova of *A. lumbricoides, Anchylostoma deodunale, Enterobius vermicularis,* ova of *Trichuris trichura* and larvae of *Strongyloides stercoralis.* Isolation of *Strongyloides stercoralis* from this previous study supports my findings in the current study of *D. melanogaster* where the same parasite was isolated. *S. stercoralis* has not been known to be transmitted though the feco- oral route, but research work has shown that there is possibility for its transmission through this route (Schmidt *et al*., 1989). There is possibility that the rhabditiform larvae once transmitted to the food substances, they undergo development to the filariform stage which then becomes infective to the host that ingests the food substance.

A previous study supportive of this *Drosophilidae* study was carried out by Hadi (2011), where he isolated three species of protozoa: *Entamoeba coli, Entamoeba histolytica* and *Iodamoeba sp*. and nematode eggs belonging to four species: *Ascaris lumbricoides*, *Ascaridia sp*, *Strongyloides sp* and *Habronema sp*. for the first time in Iraq, as transmitted mechanically by *Musca domestica*. Since *E. histolytica, Iodamoeba* and *Ascaris lumbricoides* are transmitted faeco-orally; it is feasible that these parasitic infections can be transmitted mechanically by *Musca domestica* or *Drosophila melanogaster* when these flies carrying eggs or cysts of these parasites land on food. The *Entamoeba coli* isolated in this current study is non pathogenic, however its isolation is an indicator that *D. melanogaster* is capable of transmitting other parasitic amoeboid cysts like those of *Entamoeba histolytica.* Isolation of eggs similar to those of *H. heterophyes* is interesting since the parasite's existence has so far only been reported in Egypt, North Africa and the Far East (Schmidt et al., 1989). However, the fact that these eggs were not obtained from human stool, and since eggs of other trematodes that infect birds have similar morphology, it cannot be said with certainty that these were eggs of *H. heterophyes. H. heterophyes* eggs are Small, elongated or slightly ovoidal with an operculum and a knob at posterior end. Humayun *et al.* (2002) in their study to find out the prevalence of parasites in houseflies from different areas of Lahore isolated

 

significantly high numbers of cysts and ova of different parasites from the flies. In this study the common protozoan cysts and helminthic ova detected in different insanitary and sanitary areas of Lahore were *E. histolytica, E. coli, Gia*rdia *intestinali, Iodamoeba butschlii, Ascaris lumbricoides, Ancyclostoma duodenale, Hymenolepis nana, Trichuris trichiura, Enterobius vermicularis* and *Taenia ova.* This previous study agrees with the present study where some of these ova and cysts were isolated from *D. melanogaster.*

There is a significant difference between the number of flies attracted by food and rhamnolipid compared to those attracted by water. Since food is a better attractant, it enhances the transmission of the infectious agents carried by these flies. Rhamnolipid production by *Pseudomonas aeruginosa* is an adaptive feature that brings about the attraction of *drosophilidae* for the transmission of this bacterium. Bohart *et al* (1951) in their study to investigate the filth-inhabiting flies of Guam, and also the type of bait that attracts these filth flies exposed various bait traps containing different bait types to the flies. This previous study found out that *D. melanogaster* are among the filth flies and that they are attracted to human feces, garbage and food substances. This study confirms the present study on *D. melanogaster* attraction to sweet smelling substances like fruits. It also points out to the potential of *D. melanogaster* being a mechanical vector for pathogens found in human feces. The other findings supportive of this hypothesis on fly attraction are those of Lole *et al* (2004), where *D. melanogaster and M. domestica* were attracted to food sources, putrefying and fermenting substrates emanating amines, aldehydes, ketones and alcohols; dairy products; and sugar-containing substances. This previous findings also explain the reason why some *D. melanogaster* in the current study got attracted to the *P. aeruginosa* cultures that contain 2-aminoacetophenone which are ketones. Another study previously done to investigate the food preferred by *D. melanogaster* was done by Jacob (2012). He exposed fruit flies to various food substances including sucrose solution, yeast, peptone and distilled water. It was established that the flies were attracted more to the sucrose and yeast as opposed to the peptone extract. No flies were attracted to the distilled water indicating that the fruit flies did have a preference toward the other food sources and did not randomly drink a food source.

The numbers of flies attracted by different colors were almost the same implying that *Pseudomonas aeruginosa* pigments had no effect of attracting the flies. No study has previously examined attraction of *D. melanogaster* to different colors. The present finding however, is in contrast to that by Wu *et al* (1990) who examined color attraction to another fruit fly species, *Bactrocera dorsalis*. In that study which investigated spectral sensitivity and color preference of this fly, green, yellow and orange colors were significantly more attractive than the red, black and white colors. The white color was less attractive than the red and black colors. Ravikumar *et al* (2007) did a study on attraction of different species of fruit flies to different colored traps in guava and mango orchards near Dharwad. They established that yellow and transparent traps attracted significantly high number of *B. correcta* in guava and mango. Green and orange colored traps in guava and black colored traps in mango were attractive to *B. dorsalis. B. zonata* was attracted to red colored traps in mango ecosystem. When total fruit flies irrespective of species were considered, yellow color traps were attractive in guava while black color traps in mango. The fact that our study did not show color attraction for *D. melanogaster*, our hypothesis in their transmission of *Pseudomonas* species still holds since they appear to be attracted by the smell rather than the color but still achieving the effect of attraction. It could be that the colored surfaces used here were too small to be noticed by the flies.

## 6.0. CONCLUSIONS AND RECOMMENDATIONS

*D. melanogaster* is a mechanical vector for bacterial and parasitic infectious agents of medical importance. Its ability to co-habit with *P. aeruginosa* makes these bacteria the most commonly transmitted infectious agent by the fly. *D. melanogaster* should therefore be considered as a mechanical vector of medical importance just like the housefly and the cockroach. This flies should therefore be treated like any other mechanical vectors because, they carry lots of bacteria of medical importance to food substances and also bring about infections to patients with burns. Food substances should be well covered, while fruits and vegetables should be properly washed before consumption. The burns units should also be kept free of these insects since through them, *P. aeruginosa* finds its way to the units thus compromising the recovery of patients. Insecticides should be used to eradicate these insects. Bushes should be cleared, dust bins emptied and cleaned regularly, and stagnating water drained around our residence and hospitals so as to prevent the flies from breeding. I would also like recommend an improvement on color exposure to the flies in future studies of this nature so as to be certain about whether or not *D. melanogaster* is attracted to different colors as literature suggests.

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**Fig.1**. *D. melanogaster* types (clockwise): brown eyes with black body, cinnabar eyes, sepia eyes with ebony body, vermilion eyes, white eyes, and wild-type eyes with yellow body. (Arnold, 2009).

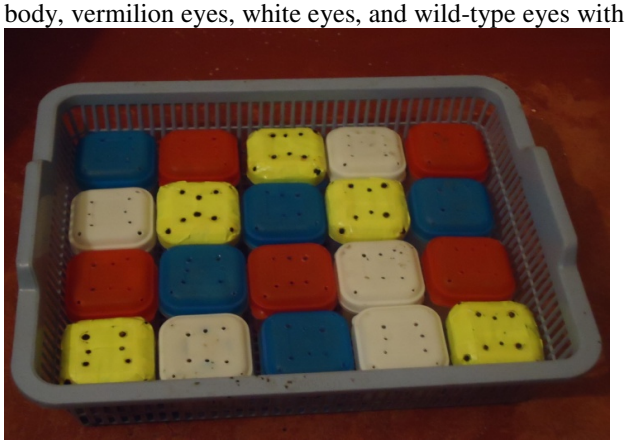

**Fig.2.** Improvised *Drosophila* trap (Wekondi, 2011).

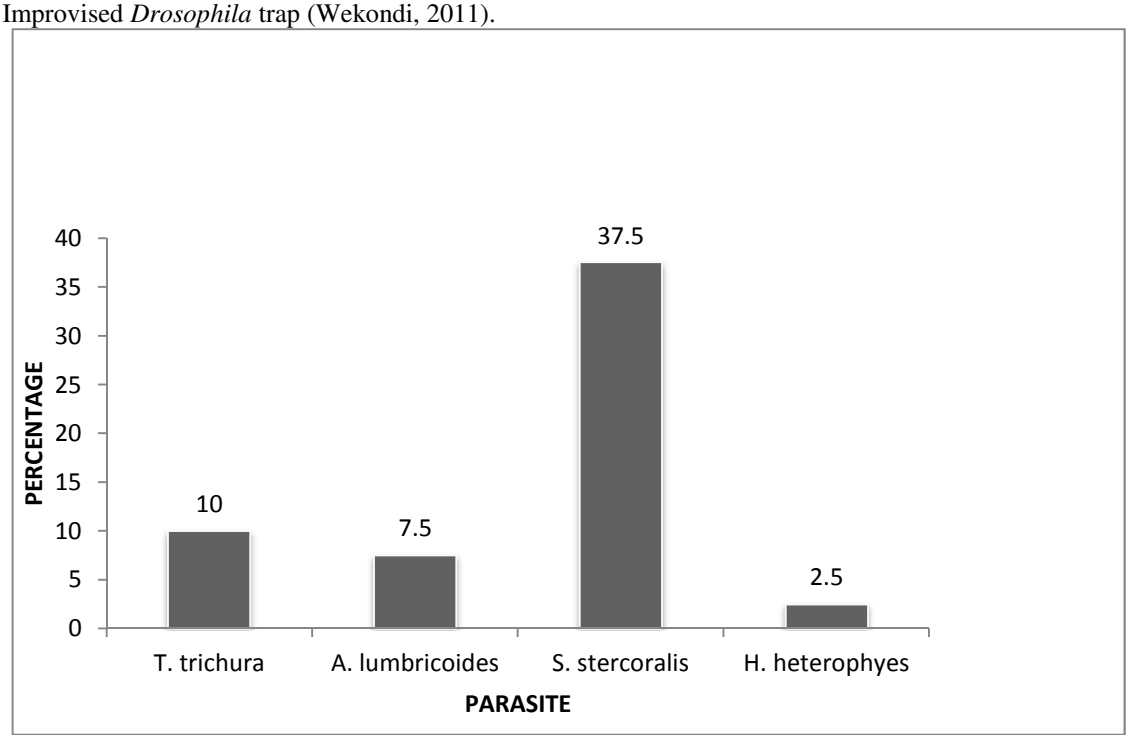


**Fig. 3:** Proportions of *D. Melanogaster* bait samples that contained parasitic infectious agents. (Wekondi, 2012).

 

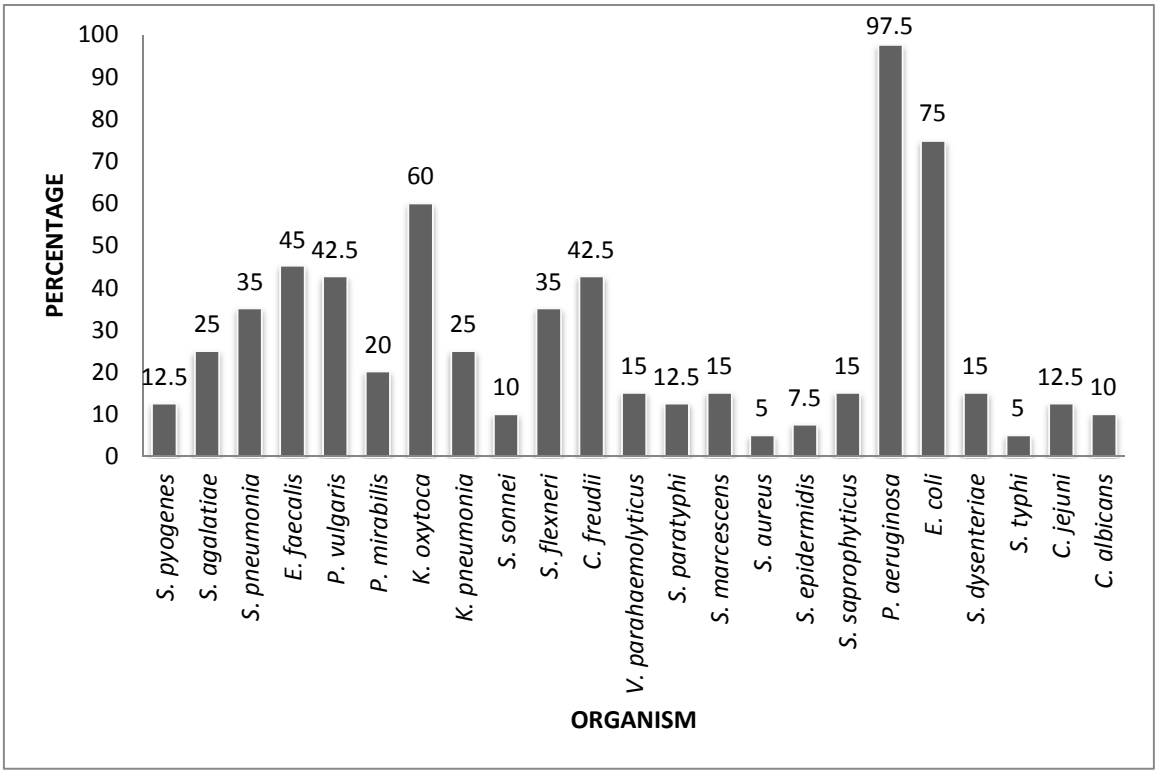


**Fig. 4:** Proportions of *D. Melanogaster* bait samples that contained bacterial and fungal infectious agents. (Wekondi, 2012).

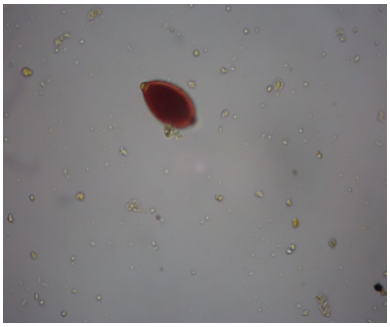

***Fig. 5***. *Trichuris trichura ova* (Wekondi, 2012).

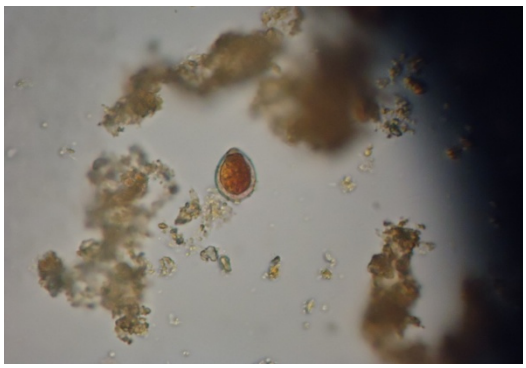

***Fig. 6***. *Heterophyes heterophyes* ova (Wekondi, 2012).

***Fig. 7***. *Strongyloides stercoralis* rhabditiform larva (Wekondi, 2012).

 

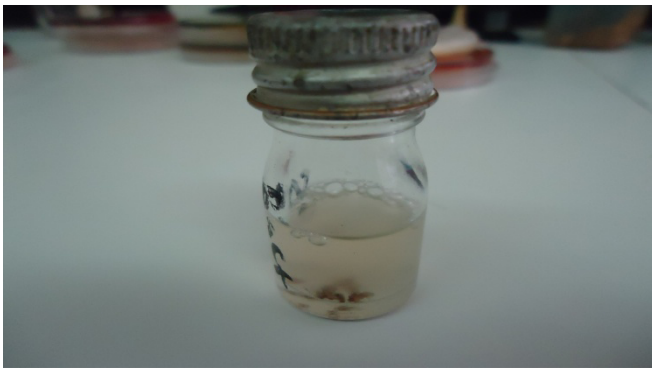

***Fig. 8***. Sample collection bottle with fruit fies in peptone water; note the turbidity of the broth (Wekondi, 2012).

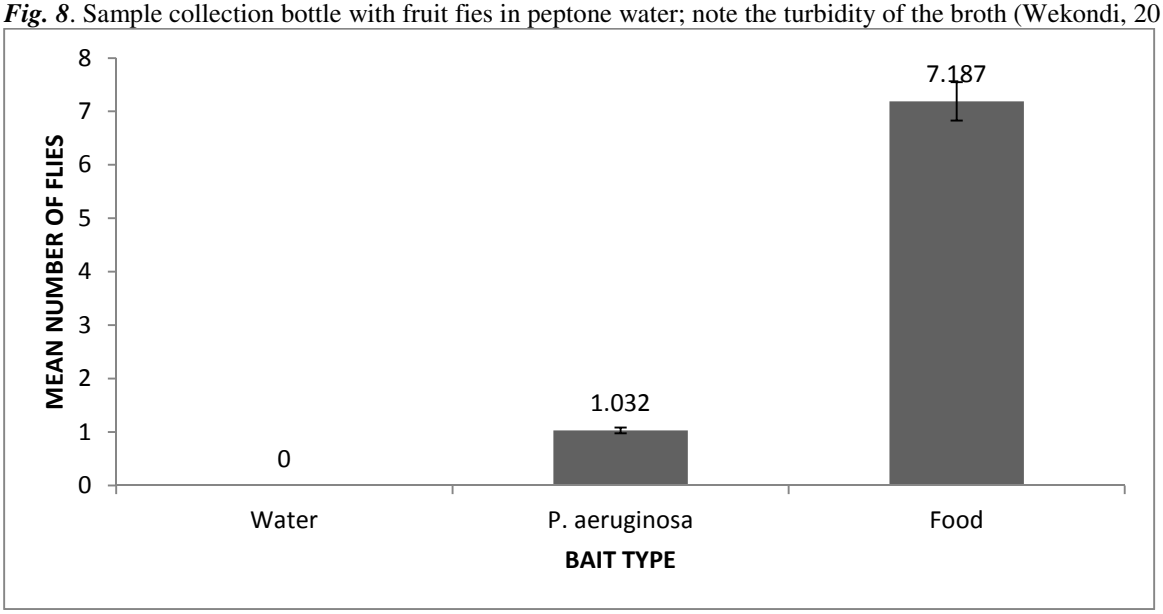


***Fig.9.*** Means of flies attracted by different bait types compared at 5% significance level.

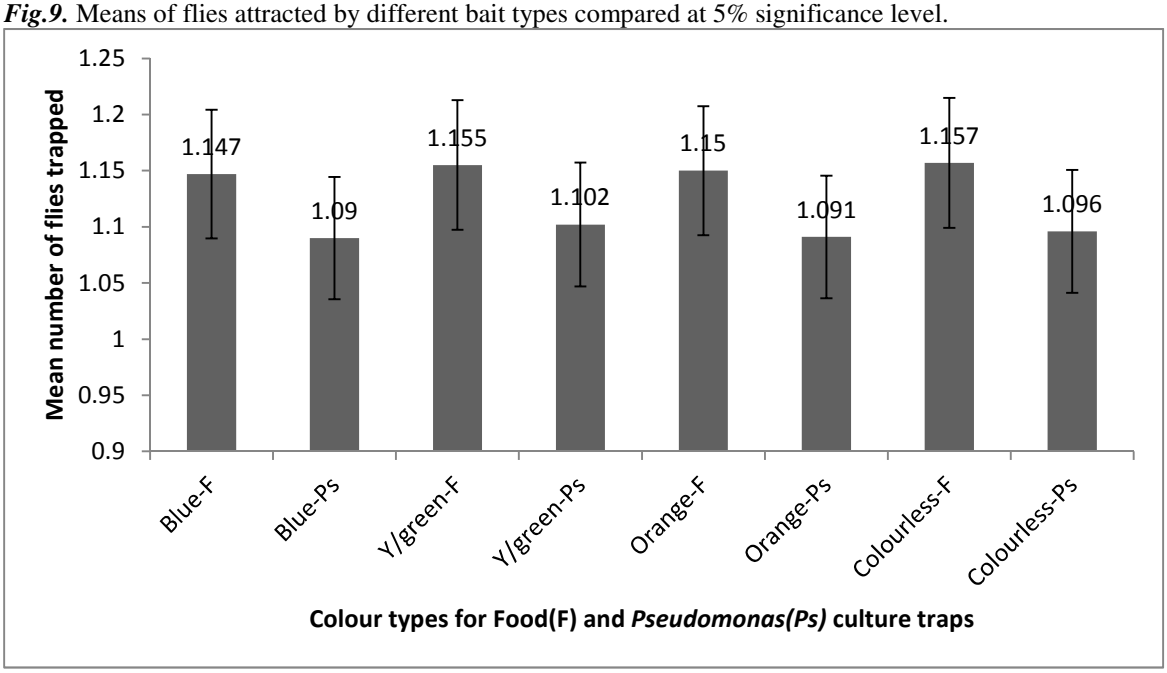


***Fig.10.*** Means of flies attracted by different color types compared at 5% significance level.

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