Global Journal of Environmental Sciences. Vol. 4, No. 2, 2005,: 131 - 134
ISSN 1596-6194. Copyright Bachudo Science Co. Ltd. Printed in Nigeria.

MICROBIAL SURVEY OF INSECT-PESTS ON REFUSE IN FIVE MAJOR TOWNS OF ONDO STATE, NIGERIA.

F. COOLBORM AKHARAIYI and F. OLUYEMI OMOYA

(Received 5 January 2004; Revision accepted 6 September, 2005)

ABSTRACT

Eight insects were identified as pests of the refuse in the five major towns in Ondo State, Nigeria. The insects are: Ant (*Psilocephala aldrichi conquillet*), Lady bird beetle (*Hyppodamia convergen*), Honey bee (*Apis mellifera*), woodroach (*Parcoblatasp*), Cockroach (*Blatta orientallis*), Housefly (*Musca domestica*), Dung beetle (*Canthon pilularis*) and Wood cricket (*Nemobius sylverstris*). From these Insects, fourteen bacteria and thirteen fungi species were recovered and identified. The internal part (gut) of the insect-pests were found to contain more bacterial load than the external part while fungal load was more on the external parts than the internal. Among the identified insect-pests, *Canthon Pilularis* was found to be more implicated of harbouring bacteria while *Nemobius sythestris* harbored more fungal load than other. *Musca domestica* had the highest population of 61.5% while *Hypodamia convergen* had the least of 0.6%.

However, the recovery of microorganisms from the insects in the surveyed refuse, highlights microbial presence and species in the environment where they were collected. There is of course the tendency of these insects contaminating foods and other materials to effect transmission of disease (s) to man, animal and plants.

KEYWORDS: Microbial, insect-pests, refuse, towns, survey.

INTRODUCTION

One of the ultimate ways to diagnose a community for a healthy condition and danger ahead of microbial infections is to study the microbial ecology as to have the knowledge of the processes that are involved in the composition, structure and diversity. Such an aspiration, however is dependent to a great extent on an adequate knowledge of the involvement and distribution of microorganisms in a community. Pathogenic microorganisms are normally transmitted by direct contact through airborne and water dispersal, through ingestion with food, and via biological vectors (Atlas and Bartha, 1998). Vectors are important in the distribution of pathogenic microorganisms that could not survive for extended periods outside host cells. Insect-pests constitute man's number one competitor for the basic survival requisite, they compete with man for his food crops, feed and fibers, his personal health. Environmental conditions often determine the distribution of pathogens, affecting host susceptibility, pathogen replication, pathogen survival, and possible routes of transmission (Atlas and Bartha, 1998). Some insects are bred in a microbe-rich environment and would have ingested and excreted saprophytic forms. Inside the insects gut some will be digested and absorbed while some persisted as injurous parasites. Among the insects not bred in microbe-rich environment, microbes commonly gained entrance to their body through the mouth as part of the food.

In a number of cases, changes in human activities have altered the population levels of pathogens or their vectors, resulting in increased likelihood of pathogen transmission to humans.

The term refuse as discussed for the purpose of this study includes garbage (waste from the storage, preparation, cooking and serving of foods) rubbish (paper, wood, plastics, leather and textile materials) Ibude, 1995). The aim of this study is to make a microbial survey on the external body and the gut system of insects of refuse dumps to evaluate their potential in contamination of foods and other materials in respect to diseases (s) transmission.

MATERIALS AND METHODS

Refuse Survey

Five major refuse dump areas were visited twice for the survey. They include: refuse around hospital or maternity, market, community refuse dumping ground, primary or secondary schools and restaurants located in Ondo, Akure, Okitipupa Owo and Ikare.

Tosses on the refuse depends on the refuse mass. Tosses were laid randomly on the sites and numbers of available insect pests on the surface refuse were counted, while dumps were gently raised to count the ones underneath. Four (4) each of the noted insects were picked with sterile forceps and the flying insects were collected with insect sweep net. The insects were immediately rinsed in different sterile universal bottles containing 2 mls of ¼ ringer's solution and were taken to the laboratory for processing. One milliliter of the ¼ ringer's solution was serially diluted. The guts of the insects were neatly and aseptically dissected out with sterile straight seekers and the contents teased out in 0.2ml of ¼ ringer's solution and also serially diluted. Aliquots (0.1ml) of the dilution were plated on each of plate count agar, Deoxycholate citrate agar, Malt extract agar and MacConkey agar. Both the spread and pour plate techniques were used for the isolation of the organisms. The ¼ ringer's solution provides a temporary isotonic medium for the microorganisms thus eliminating death of cells due to hypo or hypertonic solution. The bacterial plates cultures were incubated aerobically and anaerobically for 48 hours at 27 $\pm 1^{0}$C. Fungal plates were incubated at 27 $\pm$ 1^{0}C, for 72 hours. Meanwhile, the plates were coded with identity.

The resultant colonies at the end of incubation were counted and subcultured to obtain pure cultures. Gram staining was carried out on the subcultures to ascertain purity, which were thereafter transferred into double strength medium slants for further studies to aid identification.

F. COOLBORM AKHARAIYI, Microbiology Department, Federal University of Technology, Akure, Nigeria.
F. OLUYEMI OMOYA, Microbiology Department, Federal University of Technology, Akure, Nigeria.

Table 1: Tosses analysis of refuse insect-pests (%)

Contents	Okitipupa (n = 591)	Ondo (n = 654)	Akure (n = 622)	Owo (n =640)	Ikare (n = 637)
Paper	70 (11.8)	62 (9.5)	53 (8.5)	74 (11.6)	93 (14.6)
Leather	42 (7.1)	86 (13.1)	34 (5.5)	10 (1.6)	17 (2.7)
Food remnants	45 (7.6)	68 (10.4)	56 (9.0)	26 (4.1)	37 (5.8)
Fruits and Vegetables	20 (3.4)	32 (4.9)	68 (10.9)	84 (13.1)	75 (11.8)
Human and animal feaces	62 (10.5)	56(8.6)	75 (12.1)	54 (8.4)	68 (10.7)
Bottles	73 (12.4)	31 (4.7)	44 (7.1)	68 (10.2)	52 (8.2)
Animal carcasses	10 (1.7)	18 (2.8)	25 (4.0)	15 (2.3)	11 (1.7)
Textile materials	63 (10.7)	71 (13.6)	51 (8.2)	36 (5.6)	40 (6.3)
Wood materials	28 (4.7)	13 (2.0)	18 (2.9)	31 (4.8)	22 (3.5)
Dried leaves	64 (10.8)	88 (13.5)	77 (12.4)	86 (13.4)	97 (15.2)
Metal	98 (16.6)	95 (14.5)	87 (14.0)	95 (14.8)	68 (10.7)
Plastic	16 (2.7)	34 (5.2)	34 (5.5)	61 (9.5)	57 (8.9)

n = Total number of refuse samples identified in the tosses.

IDENTIFICATION METHODS FOR BACTERIAL ISOLATES.

a. Cultural characteristic of colonies: Colour, edge, elevation, surface

b. Physiological and biochemical characterization tests using conventional, Holt e al (1994) methods. Catalase, Motility, Gram reaction, indole, oxidative fermentation, starch hydrolysis, methylred, Voges-Proskaur tests and carbohydrate utilization.

IDENTIFICATION METHODS FOR FUNGAL ISOLATES.

Fungi were identified based on their cultural characteristics. Microscopic observation for their morphological features after Rhode and Hartman (1980).

RESULTS

Refuse Analysis

Twenty five different refuse dumps were visited twice in five major towns. The largest heap of refuse was in the community central dumping area and contained human and animal feaces, food remnants, paper textile, plastic, leather, metal materials, animal carcasses, dried leaves, polythene bags, rotten fruits and vegetables. Similar rubbish was however observed in the surveyed market areas but of lower quantity than that of the central dumping areas. The hospital/maternity environs contained paper, wood, food remnants, polythene bags and pieces of bottles. Refuse materials in the different localities were the same but more in quantity in some areas. (Table 1).

Insect Analysis

In the two visits each to the selected dumps, a total of 50 tosses were made to enumerate insect populations by extrapolation. Insect population on the dumps varied in the defferent dumps sites and in the localities. It was however observed that the more the concentration of food remnants, human and animal wastes in a refuse dump, the more was the insect population. In generality, a total insect population was estimated to be (12,859). The insect-pests founds on the refuse included: Honey bee *Apis. Mellifera* 3.05%, wood crickets (*Nemobius sylvestris)* 3.14%, Lady bird beetle Hypodamia convergen 0.59%, Woodroach (*Parcoblatta sp)* 2.52%, housefly (*Musca domestica*) 61.4%, Cockroach (*Blatta orientalis*) 2.8%, Ant (*Psilocephala aldrichii conquellet*) 25.2% Dung beetle (*Canthon pilularis)* 1.18%. (Table 2).

Microbial Population.

Both Gram positive and Gram negative pathogenic and non-pathogenic bacteria were recorded from both the internal and external parts of insect cultures incubated aerobically. Of all the representative insects selected for his study, *Canthon pilularis* had the highest internal and external total viable bacterial populations being respectively (log_{10}) 4.32, 4.87, at Okitipupa; 4.50, 4.63, at Ondo; 4.92, 4.85, at Akure; 4.86, 4.93, at Owo and 4.92, 4.79, at Ikare. The least bacterial population was recorded from the ladybird beetle. (Table 3).

The fungal populations was however not as much as that of bacterial in both the internal and external bodies of the insects. The fungal population in the insects ranged between - 0.70 to 4.38. It was observed that observed that the external parts of the insects harboured more fungal than the internal (gut) (Table 4), while bacteria were more populated in the insect guts than the external parts.

Bacteria

Fourteen different bacterial species were isolated and identified from the insect- pests during this survey. Based

Table 2: Tosses analysis of refuse insect-pests (%)

Insect	Okitipupa	Ondo	Akure	Owo	Ikare
Musca domestica	1413(11.0)	1100 (8.6)	1863(14.5)	2550(19.8)	876(6.8)=7902-(61.5)
Nemobius sylvestris	86 (0.7)	41 (0.3)	78 (0.6)	92 (0.7)	108 (0.8)= 405-(3.2)
Apis mellifera	78 (0.6)	54 (0.4)	81 (0.6)	106 (0.8)	74 (0.6) =393-(3.1)
Parcoblatta sp.	70 (0.5)	86 (0.7)	74 (0.6)	81 (0.6)	48 (0.4) =325-(2.5)
Psilocephata aldarichi onquellet	718 (5.6)	614 (4.8)	886 (6.9)	916 (7.1)	108 (0.8) = 364-(2.8)
Hypodamia convergen	20 (0.2)	15 (0.1)	25 (0.2)	10 (0.1)	6 (0.04) =76-(0.6)
Blatta orientalis	75 (0.6)	86 (0.7)	74 (0.6)	81 (0.6)	48(0.4) = 364-(2.8)
Canthon pilularis	28 (0.2)	16 (0.1)	26 (0.2)	31 (0.2)	51 (0.4 = 152-(1.2)
Total					**12,859**

Table 3: Internal and external bacterial population of insects (counts as Log 10).

	Okitipupa		Ondo		Akure		Owo		Ikare	
	Int.	Ext.	Int.	Ext.	Int.	Ext.	Int.	Ext.	Int.	Ext.
Musca domestica	4.53	4.20	4.33	4.62	4.79	1.04	4.75	4.41	4.79	4.50
Nemobius sylvestris	1.59	1.32	1.79	1.60	1.11	0.48	0.00	0.78	1.04	1.41
Apis mellfera	4.18	4.04	4.32	4.20	4.26	0.85	4.18	4.03	4.45	4.20
Percoblatta sp	4.23	1.48	4.26	1.04	1.20	0.48	4.21	1.61	1.04	0.70
Blatta orientalis	2.50	4.09	4.38	416	4.36	0.70	1.72	4.14	4.32	0.48
Psilocephata aldrichii onquellet	1.48	1.56	1.80	1.90	1.85	1.48	1.92	1.04	0.00	4.05
Hypodamia convergens	1.20	4.03	4.16	1.30	1.60	0.90	1.11	1.41	1.18	1.60
Canthon pilularis	4.82	4.87	4.50	4.63	4.92	4.85	4.86	4.93	4.92	4.79

on various conventional methods carried out on the pure cultures of each bacterial isolate and with reference to Holt et al (1994) criteria, the following organisms were associated with the insect-pests of refuse in the surveyed area: *Pseudomonas aeruginosa, Micrococcus luteus, Branhamella catarrhalis Escherichia coli, Staphylococcus aureus, Erwinia herbicola, Staphylococcus epidermidis, Staphylococcus saprophiticus, Klebsiella pneumoniae, Shigella dysenteriae, Proteus vulgaris, Salmomella typhi, Bacillus subtilis* and *Lactobacillus delbruckii.*

Fungi

The fungal organisms isolated from the insects were *Neurospora crassa, Penicillium notatum, Rhizopus stolonifer, Mucor mucedo, Botrytis cinereae, Varicosporium elodeae, Aspergillus niger, Saccharomyces cerevisiae, Candida albicans, Aspergillus fumigatus, Geotrichum albidum* and *Fusarium sp.*

DISCUSSION.

Our environment is being exposed, increasingly, to refuse contaminations from different homes, industrial set ups and commercial areas that are inhabited by insect pests most especially for food and breeding. The invasion of a habitat by microbes may be facilitated greatly by contaminated refuse and insect vectors. The uncontrolled association of microbes and insects on open refuse is one of the causes of direct and indirect contaminations that could result in microbial disease transmission to humans, plants and lower animals as well as microbial food spoilage. This connection was based on the observation that no reprehensive insect selected for this was found without microbial colonization, both on their external and internal parts. The insects with the most hairs over their bodies carried the most microbial cells on their external parts. This could be responsible for the high microbial load recorded in the dung beetle, housefly and honeybee. These insects are habitual feeders on materials with odour and sugar. Their instinct for survival and attraction to odour lead them to open prive, garbage, decomposing materials, overflowing sewage disposal units and grossly sewage-soiled areas in search of partially digested or decaying foods of their choice. By feeding on these filthy materials, the insects covered their legs, body and wings with disease-causing microorganisms. Ingraham and Ingraham and Ingraham (2002) have emphasized that when such insects land on people or their food, it spreads

Table 4:Internal and external bacterial population of insects (counts in Log 10).

	Okitipupa		Ondo		Akure		Owo		Ikare	
	Int.	**Ext.**	**Int.**	**Ext.**	**Int.**	**Ext.**	**Int.**	**Ext.**	**Int.**	**Ext.**
Musca domestica	0.70	1.11	0.85	1.00	1.70	- 0.70	1.78	4.03	1.60	4.00
Nemobius sylvestris	2.23	4.04	4.20	4.06	1.68	0.76	4.34	4.57	4.03	4.38
Apis mellfera	1.81	4.16	0.70	1.88	4.05	4.08	4.03	1.26	1.65	4.15
Percoblatta sp	0.60	-0.52	1.00	1.04	0.60	1.90	1.32	1.78	4.05	1.49
Blatta orientalis	4.19	0.71	4.14	1.65	1.04	2.01	1.49	0.00	0.60	0.60
Psilocephata aldrichii onquellet	1.49	0.78	0.70	1.00	1.04	1.86	0.48	0.04	0.70	1.28
Hypodamia convergens	0.90	4.07	4.06	4.11	0.90	4.03	- 0.40	1.11	0.48	1.04
Canthon pilularis	4.22	4.02	1.71	2.05	1.82	4.05	1.32	1.23	4.07	2.36

disease. The isolation of microorganisms from these insect-pests proved it that both crawling and flying insects can be significant agents or mechanical carriers in the spread of diseases. Insect-pests directly contaminate humans with their colonized microorganisms by depositing the organisms through feeding on ready to eat foods; and indirectly on the objects they contaminates which will serve as a transferring medium on contact by humans. In other words, they are mechanical vectors and does not cause disease (s).The medical significance of carriage of pathogenic microorganisms from refuse dumps by insect-pests depends in part upon the behaviour of the insects and in part upon the mode of transmission of these pathogens. The lady bird beetle for example is not commonly found on refuse but the few found in this study harboured a certain number of microorganisms. These insects are rarely seen in homes and rarely cause direct transmission of diseases to humans, but they can contaminate plants and field crops.

Droffner and Brinton (1995) worked on the survival of *Escherichia coli* and *Salmonella* populations in aerobic compost and observed their presence and survival even for 59 days. The isolation of these bacterial organisms from insect-pests from refuse indicates the possibility of pathogens being carried from open refuse dumps by the insects that feed on their content. The isolation of fungi from these insect-pest is also in accordance with Fungaro et al, (1996) who isolated *Metarhizium anisopliae*, an entomopathogenic hyphomycetes fungi from *Deois flavopicta*.

Some of the insect-pest identified on refuse in this study have the ability of moving long distances in search of nutrients. For this reason, it might not be accurate to believe that the majority of insects bearing these micrporganisms in their body parts originated directly form the particular refuse dump where they were found. While such contamination may arise from previous dumpsites visited, cross contamination during mating may also occur. Gregory et al (1995) have reported the recovery of sexually transmitted bacteria, virus and protozoa among mating *Adalia bipunctata* species.

It is of crucial importance to note that most of the insects identified in this study like Carthon Pilularis and Musca domestical breed on heavily contaminated materials with the potential to harbour microorganisms approximating 90%, unlike other insects which breed in less or non-contaminated materials. This occurrence can indeed facilitate microbial disease out break in areas where such is applicable.

REFERENCES

Atlas, R. M and Bartha, R., 1998. Microbial ecology Fundamentals and applications, Benjamin/Cummings Science Publishing. 4th edition. Pg. 6 –174.

Droffner, M. L. and Brinton, W. F., 1995. Survival of *E. coli* and *Salmonella* populations in aerobic, thermophilic composes measured with D.N.A gene probes. Zentralbl. Hyg Unweltmed. 195 (5): 387-389.

Fungaro, M.H.P, Viera, M.L.C, Pizzirani-Kleiner A.A and de Azevado J. L., 1996. Diversity among soil and insect isolates of *Metarhizium anisopliae var. anisopliae* detected by R.A.P.D *Letters in Applied Microbiology*. 22: 389-392.

Gregory, D.O.H., Rosie G.S., Angella, H. B., LINDA, E. W., Tamsin, M., Wilia, A. Z and Michael, E.N.M (1995). Sexual transmitted disease in a promiscuous insect, *Adalia bipunctata*. *Ecologia, Entomology*. 20: 230 - 236.

Ibude, S. O., 1995. Refuse Disposal Department of Community Health, UBTH, Benin City, Nigeria.

Ingraham, J. L and Ingraham, C. A., 2000. Introduction to microbiology 2nd edition pg. 324 Books/Cole Thompson Learning Academic Resources centre, USA.

Holt, J.G, Krieg, N.R., Sneath, P.H Stanley , J. J and Williams S. T., 1994. Bergey's Manual of Determinative Bacteriology. Wilkins Publishers. Baltimore.

Rhode, B and Hartman, G. (1980) Introducing mycology by examples. Schering. Aktiengesell Schaft Press Hambury. Pp 84-121.