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The Edibility, Distribution and Damage Indices of Oryctes Monocerosoliv. [Coleoptera: Scarabaeidae]An Edible Larva of the Oil Palms [Elaise Guineensis] and Associated Microorganisms.

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Abstract: *The edibility of Oryctesmonoceros in the Niger Delta states was established along with its methods of preparation. Its distribution showed that it flourished on Oil Palms of the States where palms are grown. Its distribution covers the whole of Niger Delta Ecological zone, Eastern and Kogi States. The damage indices of the pest in Oil Palms were also established. A microbiological assessment was done on the adult of the Oil Palm weevil, Oryctesmonoceros and this revealed the presence of some bacterial and fungal species. Studies on the larvae and piths were also done to determine the presence of bacteria in the larva, pith and adult Beetle .The pith had the highest bacterial count followed by the Internal of the larva[larva Internal], adult beetle internal (int),larva external (ext) and adult beetle external (ext). The fungal count followed the same sequential order. Total bacterial and fungal counts of the chitin of the adult beetle were* 1.3×10^7 *cfu/ml and* 1.0×10^6 *cfu/ml respectively. Four genera of bacteria and mouldswere observed respectively. Bacterial isolates identified included Bacillus, Staphylococcus, Proteus, Acinetobacter and Micrococcus species while the fungal species included Penicillium,Mucor,Fusarium and Aspergillusspecies.The implication of Staphylococcus aureus to public health is of great significance. Photograph of the edible insect is presented.*
Keywords: *Edibility, Distribution, Damage,Microbiological assessment, bacteria, fungi,Oryctesmonoceros.*

I. Introduction

Damages caused by Oryctes species pests include deformation, defoliation and destruction of parenchymatous tissues of leaves, inflorescence and stems by weevils and adults. Some insects especially members of the order Coleoptera, precisely the Oryctesmonoceros, O. rhinoceros could burrow into stems and rachis thereby creating entry points for plant pathogens that cause oil palm diseases [1]. Among the major oil palm pests in Nigeria, OryctesmonocerosOliv. (Coleoptera: Scarabaeidae, Dynastinae), O. rhinoceros, Pachymerus cardo are the most serious pests in plantations and among the Lepidoptera, the nettle caterpillar, Latoiaviridissima Holland (Lepidoptera: Limacodidae) will also be listed.Rhynchophorusphoenicis and other species are the most destructive in Raphia palms of the Niger Delta.

In an attempt to improve human protein intake by man, Animal scientists are diversifying on the domestication and commercialization of non-conventional animals such as grass cutters, snails, antelopes, zebras and other mini-livestock. In the insects research, the domestication of the Emperor moth, Bunaeaalcinoe and that of Rhynchophorusphoniencishave been presented [2,3].Considering the environmental hazards already created by large livestock on massive scale production, the livestock industries are devising means to ameliorate the hazards by introducing alternatives to conventional animals with less production cost and provide cheaper and better animal protein supply. Thus the current trend is on the insect world [4].

The larval stages of most Coleopterous pests of the Palms serve as a delicacy. The palm weevil larva which is also known as grub is harvested from palm trees infested by the adult palm weevil, which lays its eggs in the tree via natural openings or injuries.

After the palm weevil larvae (grubs) are harvested from their host palm tree, they are either eaten raw (fresh) or cooked. The cooking process is easy as only onions, salt and pepper are used as recipes. It needs no oil because the fat content of the grub is enough for its frying or grilling. Other processing/preparation methods include roasting, frying, smoking, stewing, and so on. Processed grubs are dressed on skewers for a certain amount/price and are sold in local markets, motor parks and by the sides of major roads where passers-by are attracted to purchase them.

It is also a delicacy in different parts of Nigeria where Palm trees are grown with special reference to Niger Delta, Eastern states and the West.

Among the major oil palm pests in Nigeria, the Oil PalmWeevil,OryctesmonocerosOliv. (Coleoptera: Scarabaeidae, Dynastinae) and Oryctes rhinocerosare the most serious pests of Oil Palms in the Niger Delta. Others will includePachymerus cardo which is a specialist of Palm nuts and the same time a Palm kernel borer [5]. So far there have been no reports of the distribution ofOryctesmonoceros in Oil Palm pests in Rivers State. Our objectives in this study was to assess the edibility of the beetle within the Niger Delta and other parts, its distribution and damage and to determine the microbiological quality of the palm piths where the edible larvae live, the larvae and the adult beetle.

II. Materials And Methods

Entomological Surveys

We selected four villages in Ikwerre LGA, Rivers State for the Entomological surveys In 2013. Surveys were carried out only on fallen Oil Palms. Students from the respective communities were used for the surveys. Fallen Oil Palms were opened using axes at various points of the Palm trunks. Developmental stages were harvested including the adults. The larvae were kept into open plastic bowls and covered with palm piths. The pupae were separated from the larvae and covered with palm piths as they were not feeding any more till complete metamorphosis into adulthood. The damage indices were assessed and recorded. All the stages harvested were taken to the laboratory for proper management and identification and transferred to Microbiology laboratory for relevant and further investigations.

The survey was carried out for each state of the Niger Delta, we relied on scouting for ten fallen palms per state for the collection of grubs. We randomly selected and sampled a total of ten palmsduring the rainy season of 2015. The mature larvae of O. monoceros are shown in Fig.1.

Fig. 1. Larvae of OryctesmonocerosOliv. harvested from Fallen Oil Palm Tree at Elele, Rivers State, Nigeria.

Microbiological Investigations

Adult beetles, larvae and pith from a Fallen Oil Palm treewerecollected from Rumuji in Emuoha Local Government Area of Rivers State, Nigeria. These samples were used immediately after collection. The agar used were nutrient agar (Laboratory M. Bury, Lancashire,U.K.),MacConkey agar and Potato dextrose agar [PDA] [6].

Bacterial and Fungal Viable Counts

The method used was the 10-fold dilution method of [6].Ten grams(10g) each of fresh adult beetle, larva and pithwere aseptically transferred into 90ml of sterile saline in 150ml conical flasks .The flasks were shaken vigorously to dislodge the microbial flora. Further 10-fold dilutions were carried out by adding 1.0ml of the penultimate dilution to 9ml of fresh diluents. Finally,0.1ml of an appropriate dilution was placed on dried nutrient agar, evenly spread with a sterile glass spreader and incubated at 30◦c for 24hrs.At the end of the incubation period, counts were performed for the dilutions with counts between 30 to 300 colonies [7]. All counts were performed in duplicates and the average taken.

Mould and yeast counts were enumerated by aliquots of appropriate diluted samples on acidified potato dextrose agar containing streptomycin (1mg / 100 ml) .The plates were incubated at 30◦c and counted after 48 hours for yeasts and 96 hours for moulds.

Similarly, 0.1ml of 10^{4} dilutions were inoculated on MacConkey agar. Mean colony counts were calculated and expressed as colony forming units per gram(cfu/g) of the sample analyzed [6]. Representative

colonies of the ten-fold dilution were picked and sub cultured on nutrient agar until pure cultures were obtained. The pure cultures were stored as frozen glycerol suspensions at 35◦C.These glycerol suspensions served as a means for long storage and as a source for fresh working cultures.

Identification and Characterization of Isolates: The methods described in [8] were adopted in characterization of isolates. Isolates were identified by standard methods [9].

Statistical Analysis:

Results were subjected to statistical analysis employing the student t-test at 95% probability levels.Percentage analyses were used. Also pie chart was used to show distribution and individual contribution throughout the areas assessed.

III. Results and Discussion

Entomological Surveys

Edibility: Oryctesmonoceros larvae or grubs are enjoyed as snacks in the Eastern states,Kogi state and Niger Delta of Nigeria. Table I gives the States that enjoy O. monoceros larvae as snacks and methods of Preparation.

Table 1. SelectedStates of the Niger Delta, Eastern states and Kogi StateThat ConsumedOryctesmonoceros as Snacks.

TRIBES in	EASTERN STATES	KOGI STATE	NIGER DELTA STATES		
METHODS OF PREPARATION	Raw, fried, cooked, roasted	Raw, fried, cooked, Roasted	Raw, fried, Cooked , Roasted.		
Cut open the posterior[last abdominal segment], degut, wash and add spices including onions, salt and pepper and choose to boil [cook], fry or roast.	Method of Preparation the same as illustrated and crayfish added too.	Method of Preparation is the same as illustrated	Method of Preparation is the same as illustrated, periwinkles may be added.		

In the Niger Delta, tribes that consumed O. monoceros at the rural settings include the Ikwerres. Ogonis, Okrikas, Etches, the Efiks and Oyigbos. The AkwaIboms and Calabarians eat it too. Among the Kalabaris, except for children, most people do not regard it as snacks.

The number of larvae, pupae and adults of O. monocerosharvested from fallen Oil Palm trunks in four different villages of the Ikwerre L. G. A. of Rivers State of Nigeria showed that the larval populations of O. monoceros were significantly higher than both the pupal and adults populations sampled [Fig. 2].

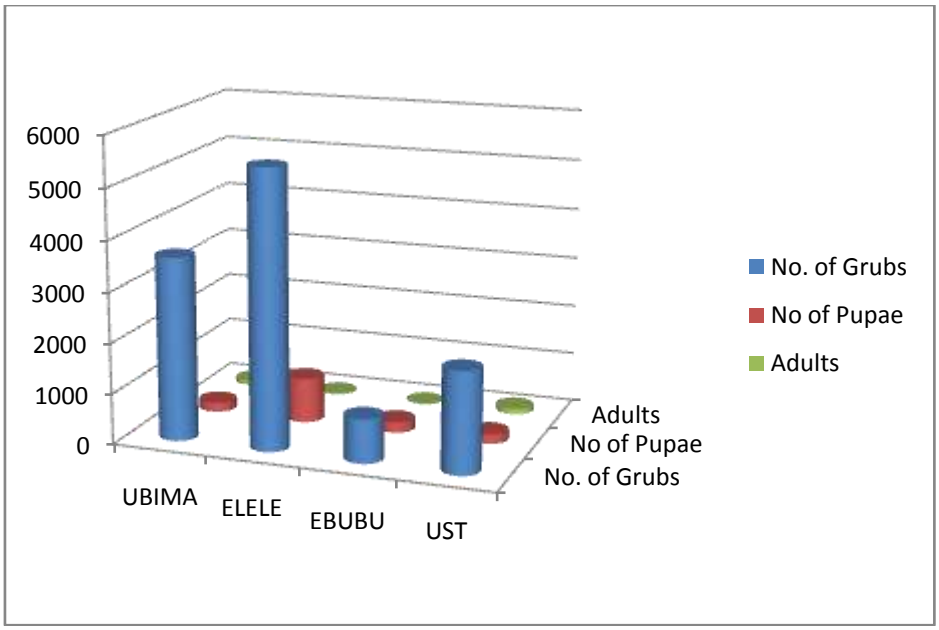
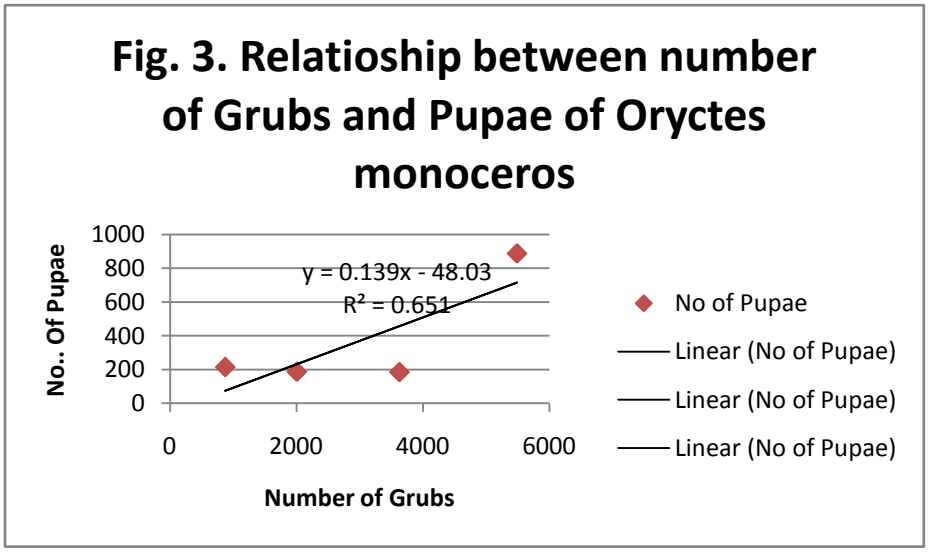


Fig.2.The distribution of the developmental stages of Oryctesmonocerosin Oil Palm Trees of Ikwerre LGA, Rivers State, Nigeria [2013].



Grubs collected from Elele were significantly higher than those from other areas[DMRT, $P<0.05$] [Fig.3]. There was a strong and positive relationship between the number of larvae and grubs of O.monoceros [y=0.1391x-48.052], as the number of larvae increased, the number of pupae also increased. The survival rate of the larvae was high despithe predation and parasitism.

Damage Indices:

Damages caused by these pests include deformationand reduction of Oil Palm Piths and trees, defoliation of leaves by the grubs and adults and destruction of parenchymatous tissues of leaves, inflorescence and stems by the grubs and adults. Some insects especially members of the Order Coleoptera could burrow into stems and rachis thereby creating entry points for plant pathogens that cause oil palm diseases [1]. Among the major oil palm pests in Nigeria, the Oil Palm weevil, Oryctesmonoceros,Oliv.and rhinoceros beetle, Oryctesrhinocerosare the most serious pests in Oil Palm plantations. The damage by Oil Palm pests mostly by the Coleopterous pests of Pachymerus cardo; Oryctesmonoceros,Rhincophorusphoenicis had been under reported in Niger Delta in particular and Nigeria as a whole. Damages caused by these pests include deformation, consumption of palm piths, defoliation and destruction of parenchymatous tissues of leaves, inflorescence and stems by the weevils and adults. Some insects especially members of the order Coleopterahave well modified mouthparts [powerfully-developed mandibles] that can chew up fibrous tissues and find their ways into the central zones of the stem [trunk]causing dead heart as they mate and lay eggs which hatch into larvae or grubs.The grubs are ableto move into any available entry holes which could be utilized by many more beetles and other exploring insects yet to attack the palm trees. They burrow into stems and rachis thereby creating entry points for other insects and plant pathogens that cause oil palm diseases [1]. The aforementioned Coleopterous insects and some Lepidopterans constitute serious pests of Palms in the Niger Delta.

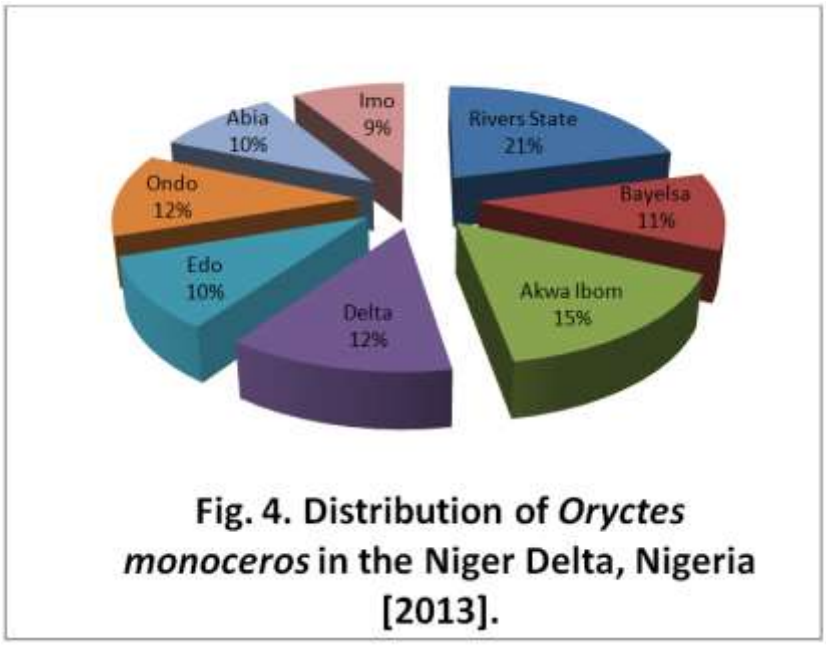


Fig. 4. Distribution of *Oryctes monoceros* in the Niger Delta, Nigeria [2013].

Fig. 4 showed the percentage distribution of O. monoceros grubs in the Niger Delta from ten Fallen Oil Palms per state. Higher number of grubs were harvested from Rivers State [21%] followed by Akwa Ibom [15%], Delta and Ondo [12%], Bayelsa [11%], Abia and Edo states [10%] and Imo [9%]. The samplers could not collect data from Cross River due to some logistic reasons. O. oryctes caused more damage in Oil Palms in states with probably higher number of palms and subsequently higher incidences as in Rivers, Ondo, Bayelsa, Akwa Ibom and Delta states. Besides, the environmental situations in the aforementioned states are very conducive for the survival, multiplication and growths of both the palms and the pests. The high humidity of the wetlands is primarily the main reason for sustainability of palm and pest populations. Edibility of the Palm grubs is not essential as rural dwellers resort to other provisions from aquatic life. However, it is a hunt in the rural settings of Imo and Abia including Anambra, Enugu, Abakaliki and some middle belts of Nigeria [10].

The total bacterial population of 1.3×10^{7} and 1.27×10^{7} cfu/ml were obtained on nutrient and Mac Conkey agar respectively. A total bacterial count of 4.49×10^{7} cfu/g was observed from an edible caterpillar of Emperor moth (Bunaeaalcinoe)[11] whereas [12] had microbial populations for the chitin of Rhynchophorusphoenicis as $9.2\text{x } 10^{5}$ cfu/ml (total heterotrophic bacteria) and in their later work [13], it was shown that a bacterial count of $1.24\text{x}10^{6}$ cfu/ml was gotten[13].Both reports of [12] and [13] were quite similar to earlier findings although the findings of [12] had less microbial counts.

The identification of Staphyloccus, Acinetobacter, Proteus, Bacillus and Escherichia was done with reference to [6]and [8].

The total fungal population of 1.0×10^{6} cfu/g was obtained on Sabroud Dextrose Agar medium. A higher fungal count of 9.5×10^{6} was observed by Braideet al.,(2011b) while Amadiet al.,(2014) had a fungal count of $7.30\text{ x}10^{5}$ cfu/ml[12] and a least fungal count of $2.0\text{x}10^{4}$ cfu/ml was obtained [13]. Four genera of moulds namely Penicillium, Fusarium, Mucor and Aspergillus species were isolated from the adult beetle. The identification of the fungal isolates were cross-matched with those described in [6] .The microbial species isolated from earlier workwere Acinetobacter, Bacillus,Klebsiella, Pseudomonas, Saccharomyces, Serratiaand Staphylococcusspp [12].

The Microbial flora observed in this study were similar to those in the works of [12 and 13, 14]. Some species that were not found in this study were found in the works of the workers [12 and 13]. Penicilliumand Aspergillus were observed in our study.

Due to the simplicity of the preparation of the grubs(larvae), taste and texture of the meat, its flavour and nutrient content, the palm weevil larva is accepted and cherished by the people of Niger Delta, south east and middle belts states. Thus it would be of great benefit to the livestock industry that the production of the grubs should be encouraged in the rural settings. More enlightenment campaigns should be made and rural dwellers should be enlightened on the production and consumption of grubs as meat to augment the shortage of animal protein in the diet of the people. Already, farmers and entomologists have embarked on the domestication of edible insects [3, 16].

Some strains of S.aureus and a species of Bacillus, B.cereus, are known enterotoxin producers [14].Their presence in these grubs used as food is of public health concern.The cooking process applied to these beetles before consumption employs temperatures that can eliminate S.aureus but not B.cereus which is an endosporeformer, hence, may withstand such temperatures. Proteus spp.are associated with high protein content foods though they rarely give rise to food borne infections but generally lower the nutritional quality of contaminated food [17]. Fungi are widely distributed in soil and air.Aspergillus spp.are frequently isolated from food. [11]. Aflatoxins produced by Aspergillus may be implicated in hepato-cellular carcinoma. Penicillium spp. produce toxins (ochratoxin-A) which is a potent nephrotoxin and causes damage in pigs and experimental animals [18]. The microbiology of edible insects and their products had been discussed [14, 19].In Africa,Asia and other parts of the world,insects and their products are used as food and feeds [20] so efforts in improving the finished products should be intensified because of the presence of the aforementioned microorganisms. Other workers [21-23] discussed the nutrient composition of O. monoceros and some discussed other pests of the palms; Rhynchophorus species.

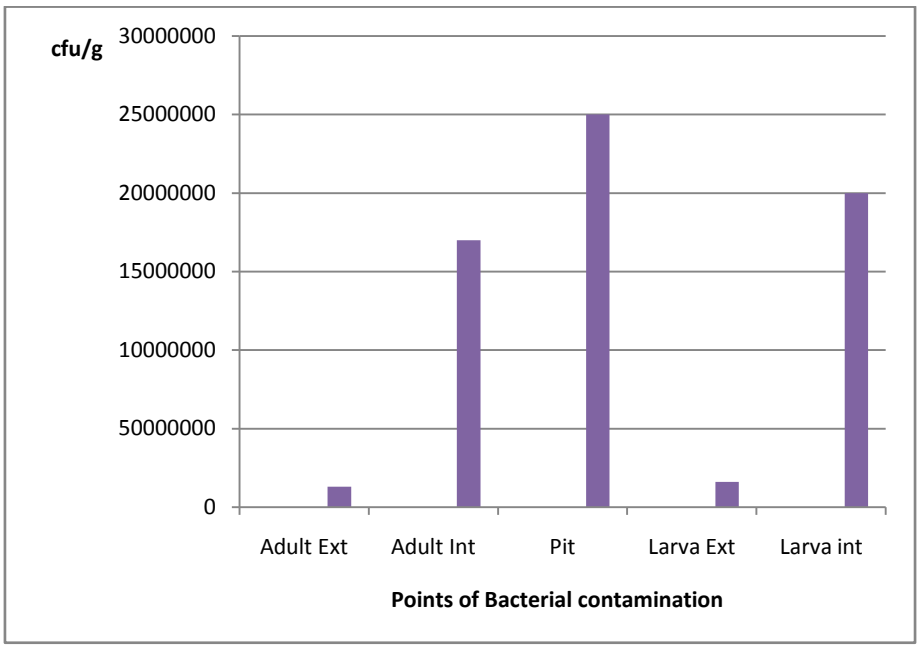


Fig. 5: Bacterial counts in different points of contamination.

A comparative study was done to observe the bacterial load present in the external of the adult, the internal of the adult, pith, the internal of the larva and the external of the larva. From Fig. 5,it is shown that the pith had the highest bacterial load. In the descending order, it is as follows: Pith>larva int>adult int>larva ext>adult ext. The fungal load showed the same pattern like that of the bacteria (Fig. 5}

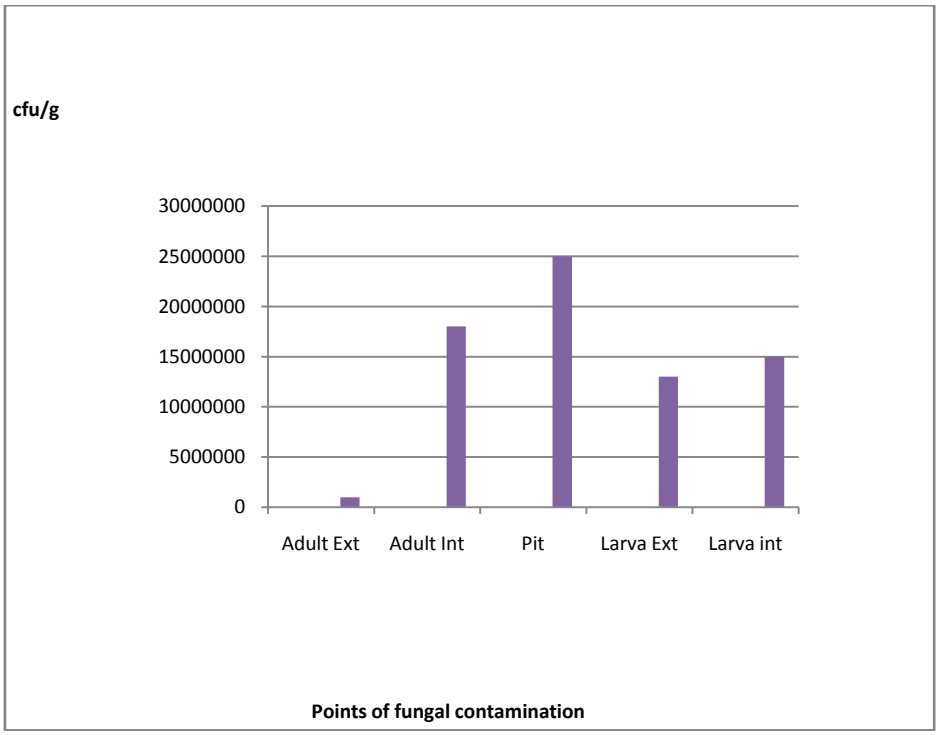


Fig. 6: Fungal counts in different points of contamination.

Analysis using the students' t-test showed that there was no significant difference at 0.05 probability levels between the bacterial and fungal loads found on the external of adult, the internal of the adult, the pith, the external and the internal of the larva. From entomological point of view, the larvae of O. monocerosnaturally feed on the palm pith and if the pith was contaminated with bacteria and fungi that explained the reasons for higher microbial loads in the internal of the larvae and the danger it poses to human health cannot be under estimated especially when some people eat them raw.What is not easily discernible is whether the pupae of O. monoceros has the same microbial contents as it pertains to its edibility; we are assessing the microbial loads of the pupae to confirm safety in its edibility.

IV. Conclusion:

O. monoceros as a supplement to diets of the rural communities who might not have access to meat and meat products due to poverty is a necessity to help augment protein as a good nutritional requirement.

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