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Concomitancy of Entomophilous and Soil Transmitted Nematodes in Selected Insects

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the occurrence of entomophilous and soil transmitted nematodes of three insect species; *Zonocerus variegatus*, *Gryllotalpa orientalis* and *Mantis religiosa* in Obio Akpor, Local Government Area of Rivers State, Nigeria.
Methodology: The insect samples were collected with entomological sweep net as described by Colwell [1] and pitfall traps as described by Davies [2]. Insect samples were dissected using Stubbins' method [3] while nematodes in the insects were isolated and identified according to Cheesborough, (2005).
Results: Two hundred and forty-eight (248) insect specimens comprising; *Z. variegatus* 193 (77.8%), *G. orientalis* 42(16.9%) and *Mantis religiosa* 13(5.2%) were collected from designated ecological settings based on availability and trapping efficiency. Out of the 248 insects sampled; 145 (58.5%) were infected with three genera of nematodes comprising; *Ascaris lumbricoides;* 17 (7.5%), *Mermis* Spp., 148 (65.5%) and *Trilabiatus lignicolus* 61(27.0%). *Mermis* Spp., an entomophilous nematode occurred in *Z. variegatus* and *Mantis religiosa* due to host specific factors. Nematode occurrence in the host did not indicate sex relationship (P>0.05) however, hosts age influenced parasite occurrence as older insects harbored more parasites (P<0.05). *Trilabiatus lignicolus*; a free-living soil nematode and *A. lumbricoides*; a soil transmitted helminthes found in the guts of *Z. variegatus* and *G orientalis* was attributed to the feeding habits of the insects.

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Conclusion: The study indicated that *Z. variegatus* and *G. orientalis* as veritable vectors of soil transmitted nematodes while *Z. variegatus* and *Mantis religiose* are suitable host of the entomophilous nematode *Mermis* Spp.

Keywords: Insect pests; entomophilous nematodes; Ascaris lumbricoides; Mermis spp.; Trilabiatus lignicolus.

1. INTRODUCTION

Insect pests constitute important limiting factors in achieving sustainable crop production and food security in sub-Saharan Africa. This stems from the fact that more than half of cultivated crops yearly are lost to insect pest infestation or diseases induced by their activities [4-8]. Although, chemical control strategies had shown reasonable results to insect pest outbreaks, however, their impact on none target organisms and the ecosystem erodes any gain derived from their field efficiency. The dangers of insecticide resistance by insect pests due to genetic modification of endemic species compounds the challenge of pest management in agriculture. Again, the elimination of non-target organisms due to incessant application of pesticides reduce or eliminate natural enemies of insect pests from their familiar environments, thereby, promoting the exponential increase in novel pest species in the environment [9,6,10,8].

Nematodes that show specificity in parasitism to insects are called entomoparasitic or entomophilous or entomogenous or entomopathogenic nematodes (EPNs).These nematodes invade specific insect hosts at variable developmental stages (larval to adult stages) as intermediate hosts and develop within their haemocoels. Active infection of the insect host by a larval stage of the nematode leads to the death of the insect host as the nematode emerges from the insect's body at maturity [11]. Although, immature stages of EPNs occur in the soil naturally as free living however, they remain obligates of adult insect hosts at the advanced stages [12,13,14]. A unique characteristic of some EPNs is their mutual relationship with Enterobacteriaceae; a highly specialized bacteria that causes mortality in insects on infection [4,15,16,14] which when liberated in the host is usually fatal. All entomophagous nematodes target insects as brood chambers, as well as, food reserves; an attribute that can be harnessed by biologist in developing biocontrol strategies to curb the over reliance on chemical pesticides in arthropod pest management in the agroecosystem [8 and 17].

In view of the fore going, it is pertinent to explore the alternative EPNs provide in the safe control of insect pests, thereby, strengthening the integrated pest management programme (IPM) in Nigeria. The suitability of EPNs as biocontrol agents of economically important insect pests must be established to ascertain the peculiarities inherent in their adoption as an alternative control strategy in the extant integrated pest management [10,8]. Studies by Dillman [18] had stated the importance of Entomophagous nematodes in the families; Steinernematidae, Mermithidae and Heterorhabditidae in the biocontrol of field insect pests; an assertion that stems from the works of Kaya and Gaugler, [4].

The funding of entomological and nematological researches in Nigeria has been the greatest bane of research and development in the agricultural and public health sectors of the country [19,20]. The prospects of using entomophilous nematodes as biocontrol agents against insect pests would enhance food production, food security and improve the gross domestic product of any country [4]. According to Grewal, [9] and Pionar [4] biological control of pests offers a better alternative in insect pests management, because, it is safe, environmentally friendly and confers long-term control on insect pests. This work sets out to determine the entomophilous nematodes species that may be specific to endemic insect species in Obio Akpor region of Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is within Obio/Akpor Local Government Area of Rivers State of Niger Delta, Nigeria which is within the metropolitan city of Port Harcourt, the Local Government Area covers an area of 260km^2 with a population of 878, 890 people according to the Nigeria National Census (2006). Geographically, Obio-Akpor is situated within latitude 4.83˚N and 6.99˚E. The sampling stations are made up of four different sites with varying plant cover types of grassland (vegetation of grasses), cultivated land (typical farmland of cereal crops), fallowed land (forest land) and a farm land for yam.

2.2 Collection of Insect Specimens

Insect samples were collected from the field by traps based on frequency of catch and species availability. Entomological sweep net of 40cm diameter aperture and 85cm depth, using the field technique as described by Colwell [1] that recommends repeated 180 degrees arc pendulum sweep, executed outwardly across the site, and returned to the starting position. Captured insects were kept in perforated plastic containers for air ventilation. Pitfall traps were used to collect *Gryllotalpa orientalis* (cricket) samples as described by Davies [2]. The traps for crickets consisted of two open-mouth (6 cm diameter and 9 cm depth) plastic containers. The traps were set-up by burying the plastic containers with each of the dug hole containing two containers of the same size. The first container was firmly locked with the excavated soil while the second container was gently inserted into the first container. The rim of the second container flushes with the ground level to avoid digging-in-effect. The second container was filled up to two-third capacity with water. A make-shift roof was constructed over the trap to prevent collection of water into the pitfall traps due to precipitation. A pinch of detergent was added to the water in the trap to serve as a surfactant. Trapped crickets were extracted from the trap by removing only the second container while the first container remained in the hole. Extracted insects were stored in properly designated air tight container and fixed with 70% ethanol.

2.3 Determination of Physical Characteristics of the Insect Samples

The weight (g) of the insect specimens was determined with an electronic scale (model: PCE-BSH 10000) while the standard and total lengths (cm) of the insects were determined using a meter rule. The standard length of the specimens involves the length of the insect without the jumping legs while the total length included the stretched-out jumping legs of the insects. The age of the insects was determined by the development or none development of the wings. The wings of the nymph stage used in the study were either under developed or none existent while the adult stages had fully developed wings [21]. Sex of the insects were determined using morphological distinctions according to Stubins [3].

2.4 Dissection and Parasitological Examination of the Insect Samples

Insect samples were dissected using Stubbins' method [3]; a drop of 70% ethanol was placed on a dissecting tray to euthanize the insect for recent catches, alternatively refrigeration was used for previously caught samples. The specimens were placed on the dissecting tray with their ventral sides facing upward. The legs of the specimen were cut off, the abdomen slightly raised before executing an incision in the middle to reveal the abdominal contents. The lateral portion of the abdominal coverings were pulled apart with the forceps and pined to the dissecting table in order to expose the digestive system which was carefully examined *in situ* with a magnifying lens (mag: x5). The processed insect was allowed to stand for a period of 20 minutes inundated with normal saline to observe any emergent nematode from the cadaver. Afterwards, the abdominal fluid and scrapings of the gut endothelium were placed on a wash glass and examined with the dissecting microscope to identify inherent nematodes. The gut endothelia scrapings and abdominal fluid were then used to prepare a saline wet mount to identify nematode or helminthes' eggs which were later stained with lugos iodine for clarity according to Cheesborough, [22].

2.5 Data Analysis

The data was analyzed with measures of central tendency and the student t-test with p-value at ≤ 0.05.

3. RESULTS

3.1 The Prevalence of Parasites in the Sampled Insects

Two hundred and forty-eight (248) insects were collected comprising; 193 (77.8%) *Z. variegatus;* 42 (17.3%); *G. orientalis* and 13 (5.2%); *M. religiosa*. Out of the 248 insects collected, 145 (58.5%) of them were infected as follows; 89.7% of *Z. variegatus*; 3.4% of *G. orientalis* and 6.9% of *M. religiosa*. Among the 193 of the *Z. variegatus* examined; 20.2% were nymphs while 79.8% were adults. Out of the 42 *G. orientalis* examined 9.5% were nymphs, 90.5% adults with 3.4% infection. Out of the thirteen (13) *M. reliogiosa* examined 15.4% were nymphs, 84.6% adults and 76% infected (Table 1.).

Table 1. Distribution of parasites amongst the sampled insects in the study

Species of insects collected	Life Stage	No Examined (%)	No Infected (%)	Total Infected (%)
Z. variegatus	Nymph	39 (20.2)	6 (4.6)	130 (89.7)
	Adult	154 (79.8)	124 (95.4)	
	Total	193 (77.8)	130 (45.4)	
G. orientalis	Nymph	4(9.5)	0	5 (3.4)
	Adult	38(90.5)	5 (13.2)	
	Total	42(17.3)	5 (11.9)	
M. religiosa	Nymph	2(15.4)	2 (20)	10 (6.9)
	Adult	11(84.6)	8 (80)	
	Total	13(5.2)	10 (76.9)	
Overall Total (%)		248	145 (58.5)	

A total of 226 nematodes species belonging to three families; Ascarididae, Mermithidae and Rhabditidae were isolated from the collected insects (Table 2) in the study. Out of this number 17(7.5%) were *A. lumbricoides*; a human geohelminth of public health importance; 148 (65.5%) were *Mermis* spp.; the entomophagous or entomophilous nematode and 61(27.0%) were *T. lignicolus*; a free-living soil nematode that is naturally associated with detritus and organic decomposition. The *Z. variegatus* had the highest parasite load of 167(75.9) followed by *G. orientalis* 42(18.6%) while *M. religiosa*; 17 (7.5%) recorded the lowest parasite load (Tables 1 and 2). Out of the 248 insects collected in the study; 155(62.5%) were females and 80 (37.5%) males with 92(63.4%) of the females and 53 (36.6%) of the males being infected (Fig. 1). There was age-related prevalence of entomophagous nematodes in the infected insects. The Nymph stage of *Z. variegatus* harbored lower load of parasites (17.9%) in comparison with the adults that recorded a parasite load of 81.8% (Table1 and 2 and Fig. 1). There was a significant difference in the infectivity of the nymph and the adult stages of *Z. variegatus* ($P<0.05$) in the study.

Table 2. Parasites speciation in the insects sampled

	Parasite speciation in the study (%)			
Species of Parasites	***Z. variegatus***	***G. orientalis***	***M. religiosa***	**Total (%)**
Ascaris spp.	6 (35.3)	11(64.7)	0	17 (7.5)
Mermis spp.	132 (89.2)	0	16 (10.8)	148(65.5)
Trilabiatus lignicolus	29 (47.5)	31(50.8)	1 (1.64)	61(27.0)
Total (%)	167 (73.9)	42 (18.58)	17 (7.5)	226(91.1)

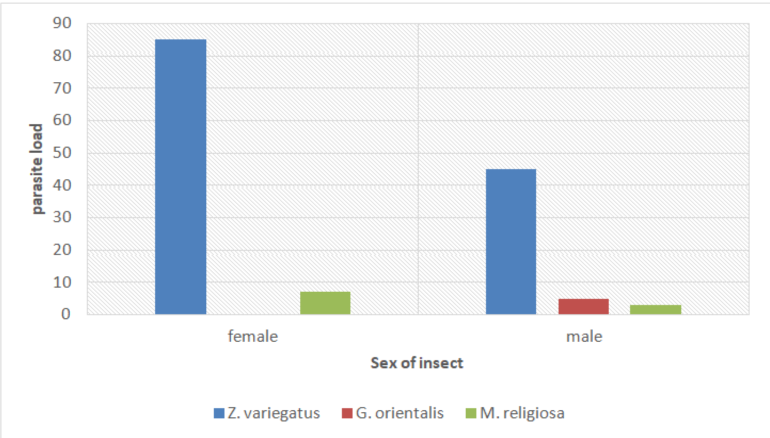

Fig. 1. Sex related Prevalence of parasites in the collected insect species

4. DISCUSSION

Amongst the insect species collected in the study, *Z. variegatus* was the most abundant, and also, harbored the highest parasite load. The abundance of *Z. variegatus* in the study area was linked to the tropical rainforest vegetation of the study area which is evergreen due to high rainfall; an ecological characteristic that promotes sustainable breeding ground for the grasshopper [10,8]. Similarly, the evergreen vegetation suited *M. religiosa*; a predatory insect that is not easily exposed because of its carnivorous solitary habit. In the study, *M. religiosa* was observed to be mainly nocturnal in habit and stalked insects that flock around light installations. *G. orientalis* is a pest to the *Dioscorea* plant (Yam); a staple crop in Nigeria, where it feeds on the underground tubers. The collection of *G. orientalis* was based on chance and trap efficiency [7]. *Z. variegatus* harbored more parasites followed by *M. religiosa* while *G. orientalis* had the least infection. Only three nematodes families were extracted from the sampled insect population, namely; Ascarididae, Mermithidae and Rhabditidae, however, the entomophilous nematode of the genus; *Mermis* spp., occurred more in the study having an overall prevalence of 148(65.5%). The occurrence of *Mermis* spp., in the insects collected was due to host specific factors that predisposed the insects to the infections. It was observed that insects with high dependence on green vegetation were mostly infected by the entomophilous nematodes; *Mermis* spp., while the soil dwelling *G. orientalis* was not infected. The lifecycle of the Mermithids involves laying of eggs on leaf surfaces where the mole cricket or any other suitable insect may not easily access them [8].

The study area is prone to repeated flooding which may impact negatively on the infectivity of nematodes generally [4]. Flooding is a natural control strategy for parasitic nematodes and effectively interferes with soil inhabiting nematodes infectivity [17]. This result agrees with many researches [4, 12, 18, 15, 16] that associated phytophagy as the point of vulnerability of suitable hosts in the entomophagous nematode parasite dynamics. The *Z. variegatus* exhibited more entomophagous nemaparasite intensity in the study, because of the phytophagous factor which exposed the insect to the preferred loci of infection where the infective juveniles of *Mermis* spp., actively invade the haemocoels of the insects.

The sizes of the insect hosts were observed to have influenced their infection by the *Mermis* spp. Thus, larger grasshoppers harbored more parasites than smaller individuals. Parasites occurrence and intensity was not strictly associated with the age of the insect hosts. The prevalence of *Mermis* species in *M. religiosa* was relatively lower than that of *Z. variegatus* [8]. The study opines that the carnivorous habit of the *M. religiosa* may have been a hindrance to it acquiring *Mermis* Spp., infection naturally [13]. This is due to the fact that the entomophagous nematode usually gains active infection through the invasion of insect hosts spiracles. However, the infection of *M. religiosa* may have been passive through the ingestion of the infected insects. *M. religiosa* may have also been actively infected by the infective juveniles of *Mermis* Spp., as they foraged on the greenery in search of hosts [23].

Mantis religiosa recorded infections in the nymph and adult stages for various parasites, but the adult stage had relatively more of *Mermi*s spp., than the other nematodes. The *Z. variegatus* had multiple infections with *A. lumbricoides* and/or *T. lignicolus* occurring in the adult stages (Table 2, Fig.1). The older *Z. variegatus*, had relatively higher parasitic load and co-infection of other nematodes. *G. orientalis* adults also, had high co infection with *A. lumbricoides* and *T. lignicolus* relative to the nymphal stages. Although more female insects were collected in the study which was a reflection of the prevalent sex ratio pattern in the environment and not due to sampling bias. Sex may not have been a determinant factor for the actual infectivity of the insects sampled [10]. The percentage of infection also skewed towards the females-based on occurrence and abundance. The recorded sex ration may be due to various factors which were outside the scope of this study. There was sex related prevalence in the study (Tables 1-3) which was significantly different ($p<0.05$).

There was a concomitancy of *A. lumbricoides* and *T. lignicolus* in the abdominal cavities of *Z. variegatus* and *G. orientalis*. This could be due to the close contact of these insects with the soil or from interferences with some aspects of anthropological activities such as open defecation, which contaminates the greenery that the phytophagous insects forage on, use of organic amendments to improve soil fertility which *T. lignicolus* acts upon and indiscriminate sewage disposal common in the study area [8]. The adult stages of *Z. varigatus* and *G. orientalis* harbored co-infections of *A. lumbricoides and T. lignicolus* (Table 1 and Fig. 1). The study opines that *Z. varigatus* and *G. orientalis* were infected with geohelminthes accidentally due to their feeding habits. This attribute nominates *Z. varigatus* and *G. orientalis* as possible agents of dispersal of geohelminths thereby, establishing a novel link in the epidemiology of geohelminths.

In view of this development, further studies need to be carried out to determine the possibility of detecting viable ova of geohelminths in the frass of insects. This is necessary considering the economic importance of *Z. variegatus* and *G. orientalis* in the study area. This study regards the occurrence of *A. lumbricoides* in *Z. variagatus* as serendipitous and should be of public health concern since, grasshoppers are reliable alternative sources of animal protein. Also, *T. lignicolus* occurrence in the collected insects indicated that the sampled insects fed on food contaminated with organic matter occasioned by human or animal excreta. The *T. lignicolus* is a good indicator of organic pollution and their presence in the gut and haemocoels of the insect species reflects the close association of the insects with debris. According to Nzeako *et al.* [17] *T. lignicolus* is a soil associated free living nematode indicative of organic pollution of both the terrestrial and aquatic habitats [6].

5. CONCLUSION

The study therefore states that entomophagous nematodes are present in *Z. variegatus* and *G. orientalis* because they are suitable hosts of the nematodes. However, the occurrence of the human geo helminth; *A. lumbricoides* ova in the mentioned insects presents a novel route of transmission of geohelminths in the epidemiology of the disease, ascariasis. The presence of *A. lumbricoides*; the human parasite for ascariasis in the gut of *Z. variegatus* and *G. orientalis* is a call for public health investigation. We therefore infer and conclude that Obio-Akpor of Rivers State is an area with a relatively high prevalence of *Mermis* spp. in *Z. variegatus* and *M. reliogiosa* and therefore could be harnessed for possible use as a biocontrol of insect pest population in the area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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