

## Research Article

# Microinvertebrates Associated with Freshly Harvested Vegetables Sold in Major Local Markets in Krobo Municipalities of Eastern Region of Ghana: Implications for Health Risks, Phytoparasite Dispersal, and Biosecurity

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**Abstract** | This study examined incidence of microinvertebrates (MIs) on freshly harvested vegetables sold across major local markets in two Krobo municipals of Eastern Region of Ghana. Cabbages, carrots, onions and lettuces were randomly obtained from different sellers in three major local markets in the study area. In repeated trials, each vegetable was incubated in the modified Baermann funnel method to extract MIs. The extracted MIs in suspensions were diagnosed and counted under a microscope, using their individual morphological features. Rotifers, ribbon worms, mites and nematodes, with uncountable zoospores of Oomycetes (i.e., egg-fungi), were associated with freshly harvested vegetables obtained from local markets. However, MIs on lettuces were more diverse than those detected on other vegetables. Nematodes were the most common groups of MIs on vegetables obtained from local markets. The majority of these nematodes belong to a bacteria-feeding group, followed by those that are crop-damaging parasitic nematodes. The genera of the crop-feeding nematodes retrieved from sampled vegetables included *Criconea*, *Scutellonema*, *Hoplolaimus*, *Pratylenchus* and *Meloidogyne*. Crop-parasitic nematodes belonging to the genus *Meloidogyne* were more common, followed by those that belong to the genus *Pratylenchus*. Implications for microparasitic pest dispersal, health risks, public education, and adoption of sanitary and phytosanitary procedures were discussed.

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## Introduction

Several microscopic invertebrates (MIs) are parasitic to livestock, humans and plants (Cui,

2014; Pechenik, 2015; Fourie *et al.*, 2017; Parker *et al.*, 2021). Those that cause parasitic diseases to livestock and humans include ribbon worms, helminthes, platyhelminthes, cestodes and mites,

among others (Cui, 2014; Pechenik, 2015; Parker *et al.*, 2021). Notwithstanding, the most ubiquitous MIs in the biosphere appear to be nematodes (Fourie *et al.*, 2017; Heve, 2021; Perry *et al.*, 2024).

Nematodes are diverse and widespread in aquatic and terrestrial ecosystems, largely because any groups of living things that belong to the three domains of life have a specific group of nematodes that parasitize them as food that sustains survival and persistence of the nematodes in habitats (Fourie *et al.*, 2017; Heve, 2021; Perry *et al.*, 2024). Thus, both aquatic and terrestrial ecosystems are enriched with individual specific groups of nematodes that are predatory on other nematodes, zooparasitic on livestock and/or humans, zooparasitic on the other invertebrates, omnivorous, and phytoparasitic on plants, among others (Pechenik, 2015; Fourie *et al.*, 2017; Heve, 2021; Heve *et al.*, 2021; Parker *et al.*, 2021; Perry *et al.*, 2024). Consequently, harmless, beneficial and harmful MIs have been in strong association with plants in the environments. For instance, plant-feeding nematodes in terrestrial ecosystems largely damage crops and cause significant economic losses to numerous crop industries across the world (Fourie *et al.*, 2017; Perry *et al.*, 2024). Many of these plant-feeding nematodes are quarantined to some specific geographical locations where they have been reported (Fourie *et al.*, 2017; EPPO, 2021; Favoreto *et al.*, 2021; Perry *et al.*, 2024). Hence, they tend to regulatorily limit access to international markets of crop produce that are largely sold in local markets, where prices of proceeds are usually very low. Often, the expansion of geographical distribution of plant parasitic nematodes worldwide have been associated with movement of plant commodities from place to place across the world (Fourie *et al.*, 2017; Knoetze *et al.*, 2017; EPPO, 2021; Favoreto *et al.*, 2021; Perry *et al.*, 2024). Thus, the correct treatments to freshly harvested plant commodities will be recommended in order to kill or get rid of any dangerous damaging parasitic microscopic invertebrates that can threaten health of livestock, humans and plants.

Unfortunately, in developing countries, lack of proper post-harvest treatment facilities coerces smallholder farmers to quickly sell off their freshly-harvested vegetables in local markets to numerous consumers. Therefore, we hypothesize that these freshly harvested vegetables sold in local markets will harbour diverse groups of MIs. Consequently, public health may be

threatened by disease-causing MIs. Similarly, in this case, further dispersal of crop-damaging parasitic microscopic nematodes to new locations may be more rapid if crop-parasitic nematode-infected freshly harvested vegetables (or the fomites) are sold in local markets to consumers who may come from distant places. In other words, improper handling and disposals of unconsumable parts of such infected vegetables may spread microscopic parasites further to distant locations. Thus, public education and awareness as well as development of biosecurity strategies will require well-investigated facts to make provisions for sanitary and phytosanitary policies. Therefore, the objective of this study was to provide empirical evidence that may lead to development of biosecurity strategies to protect the public, plants and animals for sustainable development.

## Materials and Methods

Sources of freshly harvested vegetables for the study Yilo Krobo and Lower Manya Krobo municipals in the Eastern Region of Ghana were selected for this study. Somanya market at the geographical coordinate (6° 06'18" N, 0° 00'51" W) was selected as a source of the freshly harvested vegetables from the Yilo Krobo Municipal. In the case of Lower Manya Krobo Municipal, Odumase Krobo and Kpong markets located at the geographical coordinates (5°57'31" N, 0°03'04" E) and (6°09'43" N, 0°03'29" E), respectively, were selected.

### *Sampling freshly harvested vegetables from the selected markets in Krobo municipalities*

The freshly harvested vegetables considered in this study included onion (*Allium cepa*), cabbage (*Brassica oleracea* in the *Capitata* group), lettuce (*Lactuca sativa*) and carrot (*Dacus carota*). On August 1, 2024, each of these vegetables was bought from a randomly selected vegetable seller, irrespective of whether the seller was a vegetable grower or a retailer in each of the three selected local markets. The time-length of the vegetables after the day of harvest was not necessary in this study. Moreover, the varieties of the randomly sampled vegetables were unknown or assumed to be different. Therefore, the normal freshness of the vegetables from each selected market for consumption was more important for this study. The repeated sampling of the freshly harvested vegetables from different sellers was done across the selected local markets on August 14, 2024.

### *Extraction of microscopic invertebrates from sampled freshly harvested vegetables*

The set-ups used to extract MIs are in Figure 1. The whorls of leaves of each cabbage were cut and separated from the stem. After that, the leaves and the stem of a sampled cabbage were added to a five (5) litre bowl. About 800 mL of tapwater was sprinkled over the leaves and stem of cabbage in the bowl so that MIs could migrate from the surfaces of the cabbage leaves and stem into the tapwater in the bowl, similar to the way nematodes migrate into water in a modified Baermann funnel method (Tintori *et al.*, 2022). Similar set-up was used to extract MIs, including nematodes, from freshly harvested lettuce. However, in the case of onion, about 200 mL of tapwater was sprinkled over the leaves and the stem, contained in 500 mL bowl (Figure 1). Carrot was not peeled before it was incubated in 200 mL of tapwater contained in a 500 mL bowl (Figure 1). Three (3) replicates of a set-up were made for each freshly harvested vegetable obtained from each of the three (3) local markets. The set-ups in Figure 1 were incubated for 24 hours in an open-space where temperatures varied from 27 to 35 °C. Similar approach was used to extract MIs from repeatedly sampled freshly harvested vegetables from the selected markets.



**Figure 1:** The set-ups used to extract microscopic invertebrates, with zoospores of Oomycetes, from the freshly harvested vegetables sold across selected major local markets in Yilo Krobo and Lower Manya Krobo municipalities of Eastern Region of Ghana.

### *Identification of invertebrates under a microscope*

Suspension from each bowl was examined under a microscope for incidence of any MIs. Rotifers, ribbon worms, mites and nematodes were identified according to their morphological features described in reports of Pechenik (2015), Fourie *et al.* (2017),

Heve (2021) and Perry *et al.* (2024). However, the various feeding groups of nematodes were diagnosed according to Fourie *et al.* (2017) and Heve (2021). The genera of plant-feeding nematodes were identified using the key morphological characteristics described by Smart and Nguyen (1988), Fourie *et al.* (2017) and Perry *et al.* (2024). The identified invertebrates in suspensions from incubated freshly harvested vegetables obtained from the local markets were counted. Notwithstanding, the numerous zoospores of Oomycetes (also known as egg-fungi) observed in the suspensions under a microscope were not counted.

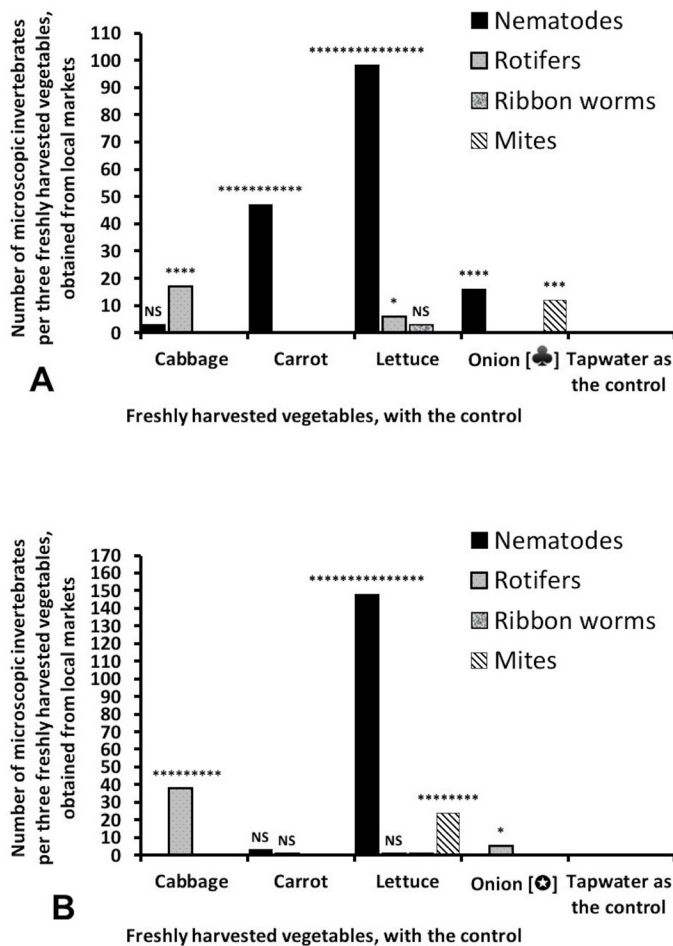
### *Data analysis*

R software [R 4.4.1~RStudio 2024.09.0-375] developed by the R Core Team (2024) for computing and/or programming was used for all statistical analyses. The numbers of individuals belonging to the various invertebrate kinds were summed up per three freshly harvested vegetables. Similar to the procedures used by Heve *et al.* (2023), Shapiro-Wilk test was used to confirm that the data for each categorical group were non-parametric i.e., not normally distributed (Gotelli and Ellison, 2013; Dinno, 2015). Hence, chi-square for goodness of fit for equal expectation was used to examine whether number of observed MIs in each categorical group was significantly larger than zero (0) at  $P \leq 0.05$ , similar to the analytical methods in reports (Gotelli and Ellison, 2013; Lente *et al.*, 2023, 2024). This was used to determine whether number of individuals belonging to a microscopic invertebrate group was actually larger than “expected zero (0) MIs on a set of three (3) freshly harvested vegetable kinds”. After that, bar graphs were used to present observations for the categorical groups.

## **Results**

### *Microscopic invertebrates associated with freshly harvested vegetables obtained from the selected major markets*

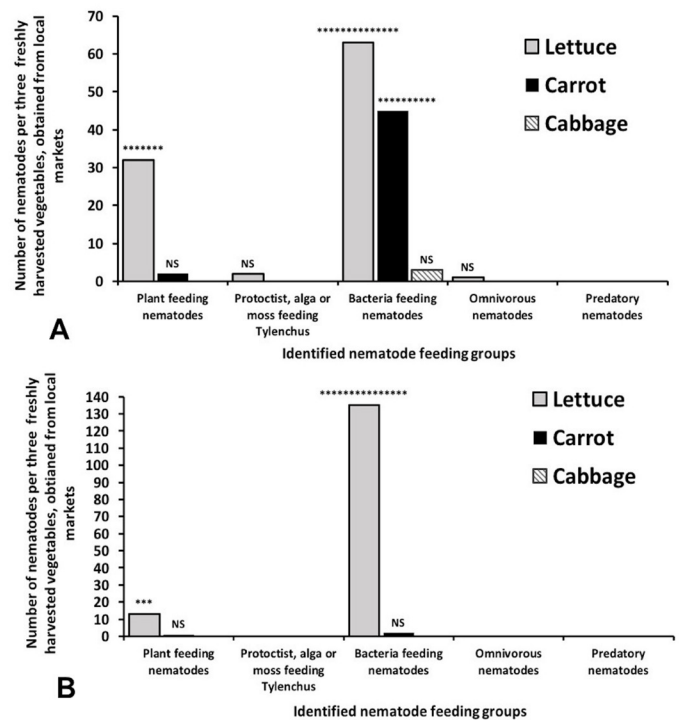
The various groups of MIs repeatedly retrieved from freshly harvested vegetables obtained from markets in the two Krobo municipals are presented in Figure 2. No MIs were found in the tapwater used as a control. However, the numbers of individuals belonging to some MI groups were significantly larger than expected zero (0) (Figure 2). In the first trial in Figure 2A, four (4) nematodes and about 17 rotifers were found on cabbages, but in the second trial in Figure 2B, cabbages had 38 rotifers, with no other MIs.



**Figure 2:** Microscopic invertebrates retrieved from freshly harvested vegetables sold in markets for the (A) first trial and (B) second trial. The symbol ‘♣’, written against ‘Onion’, denotes that all the microscopic invertebrates observed in suspensions under a microscope were dead in the first trial (A), whereas the symbol ‘⊕’, also written against ‘Onion’, denotes that few rotifers observed in suspensions were alive in the second trial (B). Chi-square for goodness-of-fit test for an equal expectation: ‘NS’ indicates that an observation was not significantly larger than expectation, being zero (0), whereas \*, \*\*, \*\*\*, \*\*\*\*, \*\*\*\*\* and \*\*\*\*\* denote that observations were significantly larger than zero at  $P \leq 0.05$ ,  $P \leq 10^{-3}$ ,  $P \leq 10^{-4}$ ,  $P \leq 10^{-8}$ ,  $P \leq 10^{-9}$ ,  $P \leq 10^{-11}$  and  $P \leq 10^{-15}$ , respectively.

Although carrots had about 47 nematodes in the first trial (Figure 2A), they had three (3) nematodes with one (1) rotifer in the second trial (Figure 2B). Lettuces had about 97 nematodes, six (6) rotifers and three (3) ribbon worms in the first trial (Figure 2A). However, about 24 mites, one (1) ribbon worm, one (1) rotifer and about 148 nematodes were found on lettuces in the second trial (Figure 2B). Onions had no rotifers and no ribbon worms, but about 12 mites and 14 nematodes found on onions were dead

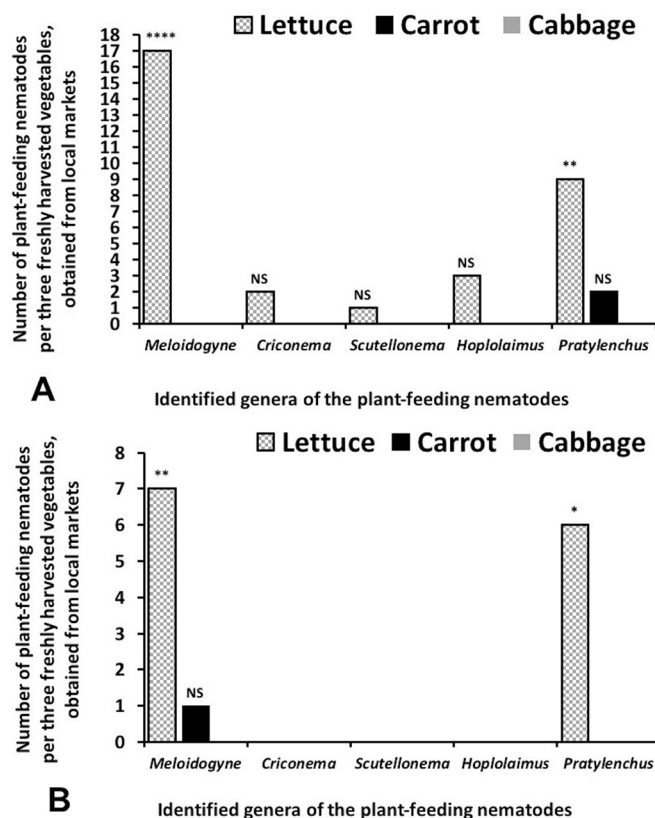
in the first trial (Figure 2A). Notwithstanding, five (5) live rotifers were retrieved from onions in the second trial (Figure 2B). In terms of numbers of live rotifers, cabbages had the highest in both trials, whereas lettuces (in Figure 2A) and carrots (in Figure 2B) had the lowest. However, cabbages (in Figure 2A) and carrots (in Figure 2B) had the lowest numbers of live nematodes, whereas carrots (in Figure 2A) and lettuces (in Figure 2A, B) had large numbers of live nematodes. Although microscopic Oomycetes are not MIs, their uncountable zoospores were observed in suspensions from all the vegetables, except onions.

[illegible]

*Nematode feeding groups associated with freshly harvested vegetables obtained from the selected major markets*

The numbers of individual MIs belonging to some nematode feeding groups were significantly larger than expected zero (0) (Figure 3). In both trials, plant feeding nematodes were found on carrots and lettuces (Figure 3A, B). Here, lettuces had the highest number of plant feeding nematodes, whereas carrots had the fewest (Figure 3A, B). Few *Tylenchus* spp.

that feed on protoctists, algae, and/or mosses were found on lettuces in the first trial only (Figure 3A). In the first trial, about 63 bacteria feeding nematodes with one (1) omnivorous nematode were found on lettuces, whereas carrots had about 43 bacteria feeding nematodes (Figure 3A). However, in the second trial, about 135 bacteria feeding nematodes were found on lettuces, whereas very few bacteria feeding nematodes were found on carrots (Figure 3B). No other feeding groups of nematodes than few bacteria feeding nematodes were found on cabbages (Figure 3A). Therefore, lettuces had the highest numbers of bacteria feeding and plant parasitic nematodes (Figure 3A, B).



**Figure 4:** Plant-feeding nematodes, belonging to the various genera, retrieved from the freshly harvested vegetables sold in selected markets for the (A) first trial and (B) second trial. Chi-square for goodness-of-fit test for an equal expectation: 'NS' implies that an observation was **not** significantly larger than zero (0), whereas \*, \*\* and \*\*\* indicate that observations were significantly larger than zero (0) at  $P \leq 0.05$ ,  $P \leq 10^{-2}$  and  $P \leq 10^{-4}$ , respectively.

#### Plant parasitic nematodes associated with freshly harvested vegetables sold in the selected markets

Plant feeding nematodes belonging to the various identified genera found on freshly harvested vegetables sold in selected markets in the two

Krobo municipalities are presented in Figure 4. One (1), two (2), three (3), nine (9) and 17 individual plant-feeding nematodes belonging to the genera *Scutellonema*, *Criconema*, *Hoplolaimus*, *Pratylenchus* and *Meloidogyne*, respectively, were found on lettuces in the first trial (Figure 4A). Also, carrots had two (2) individual plant feeding nematodes that belong to the genus *Pratylenchus* only in the first trial (Figure 4A), but no plant-feeding nematode was found on cabbages in both trials (Figures 3 and 4). In the second trial (Figure 4B), six (6) and seven (7) individual plant feeding nematodes belonging to the genera *Pratylenchus* and *Meloidogyne*, respectively, were found on lettuces, whereas only one (1) individual belonging to *Meloidogyne* species was found on carrots. Therefore, lettuces had five (5) different genera of plant feeding nematodes more than other vegetables had (Figure 4).

## Discussion

### Variation in incidence of microscopic invertebrates associated with vegetables from local markets

Several factors such as the ecology of plants and farmers' practices may contribute to make diverse MIs to associate themselves with vegetables from fields to markets, where they are sold to consumers. The freshly harvested vegetables randomly obtained from local markets could be assumed to have gone through unpredictable different conditions in the field and/or during harvesting, bagging, storage and transportation to the local markets. Consequently, these vegetables from the local markets would have had exposure to unquantifiable unequal conditional treatments that could vary incidence of MIs on them. Nonetheless, lettuces from the local markets in the study area were harbouring more diverse MIs, with nematodes being the most abundant. Lettuce's leaf structure may retain moisture more effectively than many other vegetables may do. Thus, farmers' irrigated crops that have dense foliage and high humidity levels will often provide ampler habitats to accommodate and support diverse microfaunal communities (Dhooria, 2016; Dastogeer et al., 2020). Moreover, in this study, the majority of the harvested lettuces had roots with some amount of soil adhered to them. An agricultural soil is a potential medium that harbours diverse MIs (Pechenik, 2015; Perry et al., 2024). Soils around the roots of harvested vegetables in the modified Baermann funnel-like set-ups could also be the cause of large numbers of MIs associated with lettuces. However, we observed that almost all the MIs associated with onions could

hardly survive. This could be attributed to the fact that onions contain allicin and other antimicrobial compounds that might possibly suppress plant-colonizing MIs on onions (Sharma *et al.*, 2018; Choo *et al.*, 2020)

#### *Implication for health risks to animals and humans*

Parasitic ribbon worms such as acanthocephalans are parasites that infect fish, livestock and humans (Pechenik, 2015). These worms are less common than other parasitic invertebrates. *Carcinonemertes epialti* preys on eggs of infected egg-bearing crabs and other animals in marshy or aquatic habitats (Pechenik, 2015). In this case, ribbon worms can sometimes pose health risks to livestock and humans if infected hosts such as crabs and other ovigerous hosts are not properly cooked for meals.

Several bacteria have been isolated from the cuticle and gut of nematodes, proving that nematodes are largely in phoretic association with several prokaryotes (Campos-Herrera, 2015; Topalovic *et al.*, 2019; Ogier *et al.*, 2020). Unfortunately, some of the retrieved bacteria from nematodes belong to the genera of pathogenic prokaryotes that cause disease in arthropods, livestock, humans and other animals (Dunker *et al.*, 2013; Topalovic *et al.*, 2019; Ogier *et al.*, 2020). Perhaps, other MIs observed in this study may be in similar phoretic associations with pathogenic bacteria that may likely attack pets, livestock and humans, among others. Thus, such phoretic interactions between MIs and disease-causing pathogenic bacteria can have health risks to animals and humans. Meanwhile, certain zooparasitic nematodes such as strongylids (which cause strongyloidiasis) and soil-borne eggs of *Ascaris* spp. are potentially infectious to livestock, pets and humans through eating contaminated vegetables (De Silva *et al.*, 2003; Hotez *et al.*, 2007; FAO, 2016; WHO, 2020).

#### *Implication for plant health risks and phytoparasite dispersal*

The majority of crop damaging nematodes are soil-borne parasites; they are very difficult to be controlled using pesticides, because they are shielded in soil (Perry *et al.*, 2024). In this study, we observed different genera of plant parasitic nematodes in association with the selected freshly harvested vegetables obtained from local markets. Moreover, the individual plant-feeding nematodes belonging to *Meloidogyne* were

the most dominant, followed by those of *Pratylenchus*. Unfortunately, every *Meloidogyne* species with *Pratylenchus* species is known to be the first top world-ranked crop-damaging parasite among several economically important plant-feeding nematode groups (Jones *et al.*, 2013; Fourie *et al.*, 2017; Perry *et al.*, 2024). Moreover, movement of numerous plant-feeding nematodes, including some species belonging to the genus *Meloidogyne*, is regulated across the world (Fourie *et al.*, 2017; Perry *et al.*, 2024). Consequently, plant commodities, with which these economically important plant parasitic nematodes are associated, are largely regulated across the world (Jones *et al.*, 2013; Knoetze *et al.*, 2017). Unfortunately, in developing countries, movement of plant commodities, including that of freshly harvested vegetables, from one region to another is not regulated to minimize the spread of plant-parasitic nematodes to distant locations within the country. From our view, live plant parasitic nematodes associated with freshly harvested vegetables from local markets in the study area can be spread further to other parts of Ghana and beyond if the vegetables are sold to consumers or buyers who may transport the crops to several distant locations across the country.

#### *Implication for biosecurity measures*

Observations in the current study suggest the need for sanitary and phytosanitary (SPS) policies, although similar studies may be required across the major markets in Ghana. Regulatory measures will ensure effective biosecurity across markets in Ghana. This is because biosecurity strategies are generally aimed at protecting plants, the public, animals, pets and livestock, among others against pathogens and parasites that are associated with food and plant commodities (Boehm *et al.*, 2017; Kang and Ramizo, 2017). In countries where SPS measures are well adopted, the majority of the technical barriers that restrict access to international markets for plant commodities have been removed (Kang and Ramizo, 2017). Therefore, through the adoption of SPS policies, local vegetable industries in the study area may have access to international markets for sustainable development.

## Conclusions and Recommendations

Rotifers, ribbon worms, mites and nematodes, with uncountable zoospores of Oomycetes, were the MIs associated with freshly harvested onions, cabbages,

carrots and lettuces sold in major local markets in two Krobo municipals. However, MIs that were associated with lettuces were more diverse than other vegetables had. Nematodes were the most common group of MIs associated with the vegetables sold in local markets. The majority of these nematodes belong to a bacteria-feeding group, followed by those that are plant-parasitic on crops. The genera of the crop-damaging nematodes retrieved from the freshly harvested vegetables obtained from local markets included *Criconea*, *Scutellonema*, *Hoplolaimus*, *Pratylenchus* and *Meloidogyne*. Individual MIs belonging to the genus *Meloidogyne* were the most common crop-parasitic nematodes, followed by those that belong to the genus *Pratylenchus*. Implications for dispersal of parasitic invertebrate pests and the health risks associated with MIs found on vegetables sold in local markets have been discussed. Therefore, recommendations for public education and adoption of sanitary and phytosanitary measures to achieve sustainable development have been suggested.

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## Novelty Statement

This study tends to create awareness about diverse live microinvertebrates on plant commodities from local markets. The majority of these MIs were live plant parasitic nematodes associated with freshly harvested vegetables, which were sold in the selected markets. Thus, effective sanitary and phytosanitary (SPS) policies and procedures are required at all levels of 'vegetable supply chain' to reduce the health risks and dispersal of live animal or plant parasitic ones.

## Author's Contribution

William K. Heve (PhD) conceptualized the study, supervised the collection of data, diagnosed the various groups of microinvertebrates (including nematodes) in suspensions, analyzed the data using R software, wrote the manuscript and carefully edited it for publication.

Paul O. Aniapam collected samples of freshly harvested vegetables from local markets and then set up the modified Baermann funnel method to extract microinvertebrates into suspensions, under the auspices of William K. Heve (PhD).

## Data availability statement

Information collected during this study is included in this article. However, data will be available upon request.

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This study received no grant and no support from any commercial institutions, funding agencies in the not-for-profit, or public sector. Nonetheless, the authors contributed their small resources to fund this study.

## Human and animal rights

The authors did not use animal or human bodies as objects or materials for this study.

## Conflict of interest

The authors have no conflicting interest to declare.

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