Faculty of Natural and Applied Sciences Journal of Scientific Innovations
Print ISSN: 2814-0877 e-ISSN: 2814-0923
www.fnasjournals.com
Volume 4; Issue 1; March 2023; Page No. 182-192.

PARASITIC ANALYSIS OF THE RHIZOSPHERE AROUND THE ROOTS OF BITTER LEAF (*Vernonia amygdalina*) GROWN IN OBIO/AKPOR LOCAL GOVERNMENT AREA RIVERS STATE NIGERIA

***Elele, K., Anwuri, N., & Ogbuku, C.P.**
Department of Biology, Ignatius Ajuru University of Education, Rumuolumeni Port Harcourt, Nigeria

***Corresponding Author (Email): kingsley.elele@gmail.com**

Abstract
This Research Work on the parasitic analysis of the rhizosphere around the roots of the Bitter leaf (*Vernonia Amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria, was conducted over six weeks from July to August 2022. A total of 30 soil samples (10 from each location) were collected from three communities; Rumuolumeni, Ogbogoro and Rukpokwu and examined using the modified Baermann's technique for nematode extraction. The results revealed that 24(80%) of the soil samples were infected with PPN's. Three genera of plant parasitic nematodes were encountered in the experimental setups and recorded as follows: *Criconemella* spp., 9(30%), *Meloidogyne* spp., 8(26.7%) with the least occurrence coming from *Pratylenchus* spp. 7(23.3%). Further analysis showed that samples from all three communities were equally infected 8(80%). However, the number of nematodes recovered from each community varied and are presented in descending order as follows: Rumuolumeni 92, Ogbogoro 67 and Rukpokwu 52. Chi-square statistics show that the relationship between community and infection was non-significant ($p>0.05$). This result revealed a very high infection rate of plant parasitic nematodes in the three communities in the Obio/Akpor Local Government Area. It is therefore recommended that adequate efforts should be put in place by the government to provide nematicides to the farmers and make them available to both commercial and peasant farmers and serious awareness of the implications of these plant parasitic nematodes should be carried out regularly in order reduce the burden of plant parasitic nematodes as well as curtail economic losses due to these parasites.

Keywords: Rhizosphere, Bitterleaf, Nematodes, Obio/Akpor, Roots, Parasite

Introduction
The consumption of vegetables has increased in recent years because of their nutritional importance, health benefits and constituent of a balance (healthy) diet (Pem & Jeewon, 2015). A diet rich in vegetables and fruits can lower blood pressure, reduce the risk of heart disease and stroke, prevent some types of cancer, lower the risk of eye and digestive problems, and have a positive effect on blood sugar, which can help keep the appetite in check. Eating non-starchy vegetables and fruits like apples, pears, and green leafy vegetables may even promote weight loss (Bertoia et al., 2015). Vegetables are also vital sources of energy that humans depend on as food supplements or nutrients (Taha et al., 2018; Alhabbal, 2015). *Vernonia amygdalina* commonly called bitter leaf or Onugbu in Eastern Nigeria among Igbo and Igboid languages. This vegetable is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs. *V. amygdalina*, a member of the family Asteraceae, is a widely used vegetable that can grow in different parts of the world (Michel et al., 2020). *V. amygdalina* is frequently found in gardens and can adapt to a variety of climatic conditions, unlike other plants. *V. amygdalina* occurs naturally along rivers and lakes, in woodland and grassland up to 2800 m, and in regions where the mean annual rainfall is 750-2000mm (Ifeoluwa et al., 2017; Oseni & Babatunde, 2016). It requires full sunlight and grows in a humid environment. It can also grow on all soil types but *V. amygdalina* can be commonly found along drainages and in natural forests or at home and commercial farms

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations*,*4*(1), 182-192.

and is best planted on humus-rich soils (Ifeoluwa et al., 2017). Although it is popularly used for food, it has also, been traditionally used for medicinal purposes (Biressaw & Birhanu, 2017). Cooked leaves of *V. amygdalina* are a staple vegetable in soups and stews of various cultures throughout Nigeria. It is known as Ewuro in Yoruba, Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo) and Chusar-doki (Hausa). The leaves are green in colouration and have a bitter taste. Their traditional use is not only limited to humans alone as it is added to horse feed to provide a strengthening or fattening tonic known as 'Chusan Dokin' in Northern Nigeria (Oseni & Babatunde, 2016). *Vernonia amygdalina* Del is among the medicinal plants used for the treatment of various diseases in different parts of the world. It has been reported that *V. amygdalina* Del plant is used for the treatment of diabetes, yellow fever, dysentery, constipation, malaria and stomach ache in Africa and Asia (Adegbite & Sanyaolu, 2009; Ebong et al., 2008). Moreover, the plant is widely used in Africa as a source of food as a vegetable and culinary herb in soup (Nwosu et al., 2013). *V. amygdalina* Del belongs to the family Asteraceae. It is widely distributed throughout Tropical Africa and cultivated as a food supplement in West Africa including Nigeria (Adegbite & Sanyaolu, 2009; Atangwho et al., 2009). The common name of *V. amygdalina* Del is 'bitter leaf', locally known as 'Shuwaka' in Hausa Language and 'Ewuro' in Yoruba Language (Farombi, 2011).

According to WHO, some parasites, such as fish-borne trematodes, are only transmitted through food. Others, for example, tapeworms like *Echinococcus spp*, or *Taenia solium*, may infect people through food or direct contact with animals. Other parasites, such as *Ascaris, Cryptosporidium, Entamoeba histolytica* or *Giardia*, enter the food chain via water or soil and can contaminate fresh produce. Parasitic infections are mostly spread by human activities which may include: Poor personal hygiene and consumption of unwashed or poorly washed vegetables and fruits (Adams & Motarjemi, 2017). Intestinal parasites that are common in raw vegetables include protozoa, nematodes, cestodes, and trematodes (Umeanaeto et al., 2016). There is high consumption of vegetables especially bitter leaf (Onugbu) in the rural part of Nigeria. This may be associated with its health benefits.

Vernonia amydgalina is rich in nutrients and phytochemicals and this invariable accounts for its positive influence on health. Compounds associated with its anticancer effects, antioxidant properties, antimalarial properties, anti-inflammatory properties, antimicrobial properties and hypolipidaemic effects have been identified but most mechanisms of action have not been elucidated in humans. Research has also shown that the metabolites in the leaf have specifically been efficacious against parasites, especially worms. The mechanisms of activities include paralysis of the worm, interference with energy generation and impairment in nutrient absorption, motility, and reproduction (Ifeoluwa et al., 2017). *V. amygdalina* is used in traditional herbal medicine. This includes alleviating malnutrition that may be due to micronutrient deficiencies, useful in the treatments for diabetes, lowering cholesterol and is used in the treatment of infections (Ojimelukwe & Amaechi, 2019). They are eaten raw or mashed to get the juice used in treating abdominal difficulties such as diarrhoea, stomach upset, and gastrointestinal tract diseases like dysentery and related other issues. They are also eaten after de-bettering through normal squeeze-washing.

The presence of nematode populations puts agricultural production in Africa at a significant risk, given the fact that most farmers do not know the actual nematode specie present in their farms. Root-knot nematode is a major pathogen of fruits, vegetables and other food crops in different parts of the world. The short lifecycle of 6 to 8 weeks enables root-knot nematode population to survive well in the presence of a suitable host and their population builds up to maximum usually as crops reach maturity (Shurleff, 2000). The recent classification of nematode is based on the hypothesis of Paramonov and Filipjev (Shah & Mahamood, 2017). About 50 million nematodes are found in a square meter of moderately fertile soil to 30 cm depth. Nathan Augustus Cobb, father of nematology provided a good mental picture of the importance and diversity of nematode when he stated that, "if all matter in the universe except nematode were swept away, our worldwide still be dimly recognizable We should find its mountains, hills, valley, rivers, lake, oceans represented by a fill of nematodes" (Mandal et al., 2021). Nematodes fit in the category of Ecdysozoa that constitute animals which can moult their cuticle. More

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations,4*(1), 182-192.

than 30,000 species of roundworm are found in nematodes generally in the range of 0.2 mm to 6 m. An agriculturally important species of plant parasite nematode called Root-knot nematodes were recognized by Barkeley in galls on cucumber roots (Sterling et al., 2013; Alhabbal, 2015; Regmi et al., 2016). Nematodes are familiar with all types of soil and environments worldwide and are found in the maze of interconnected through called pores which are formed by the soil formation process (Guerena, 2006; Sterling et al., 2013).

Plant Parasitic Nematodes (PPN) are small worm-like transparent, bilateral symmetry, pseudocoelomate, multicellular, free-living or parasitic microorganisms which are predatory, aquatic, terrestrial, entopathogenic, ectoparasite, endoparasite, semi-endoparasite i.e. (e.g. *Tylenchulus semipenetrans*) or sedentary (Ozberk et al., 2011; Shah & Mahamood, 2017). PPN are prolonged, slender organism that has glistering smooth surfaces Young one has body tapering to a point towards both ends but adults are swollen and no longer resemble worms (Kumar & Yadav, 2020) and (Goss, 2008; Okeke et al., 2022). Plant parasite nematodes are identified by stylet and sub-ventral and dorsal oesophagus glands which play a significant role in evolutionary adaptations for plant parasitism. Due to the numerous annulations, they look segmented on the cuticle that allows bending without kinking but is unsegmented. Their bodies have identified organs for feeding, digestive, nervous and excretory systems and have a well-developed reproductive system which lacks circulatory and respiratory organs (Goss, 2008). Most of the species are called "farmers' best friends because many species cause death to insects" (Shah & Mahamood, 2017). Crop damage from nematodes is not readily apparent in most cases, and it often remains hidden by the many other factors limiting plant growth (Ami & Taher, 2013; Mukhtar et al., 2018). Nematode management should be multifaceted. Since eliminating nematodes is not possible, the goal is to manage their population, reducing their numbers below damaging levels (Bauters et al). Common management methods used include planting resistant crop varieties, rotating crops, incorporating soil amendments, and applying pesticides. In some cases, soil solarization also may be practical (Gozel et al., 2006; Bekele et al., 2017). Despite the health benefits of the leafy vegetable under study, little or no information exist on the possibility of it being a source of infection to the consumer through contamination. The culture of inadequate or poor washing of vegetables could pose significant and major health threats to the consumers who eat this vegetable raw sometimes, especially when they squeeze the leaves manually to extract the juice and drink the same to prevent or cure one ailment or the other.

Pratylenchus spp is distributed throughout the world. They belong to genus Pratylenchus constituting about 97 valid species. Major host ranges are cereals legumes vegetables, fruits, ornamentals, coffee, peanuts etc. It is estimated that 8 species of root lesion nematode cause devastating damage to cereals crop (Yu et al., 2012; Subedi et al., 2020). Specific symptoms associated with the vegetable crop plant are that it causes yellowing of foliage, reduction in growth and necrosis in the root and tuber of potato (Verdejo-Lucas & McKenry, 2004; Esteves et al., 2015). They are migratory endoparasitic inhabitants. The life cycle last longer than 3-9 weeks depending on species and environmental conditions. For example, in Red Clover, the life cycle duration of *P. penetrans* is 9 weeks. The modes of reproduction are parthenogenesis and anhydrobiosis (Jones & Fosu-Nyarko, 2014). Therefore, this study was carried out to investigate the parasitic analysis of the rhizosphere around the roots of bitter leaf (*Vernonia amygdalina*) grown in Obio/Akpor Local Government Area of Rivers State Nigeria.

This study will be of immense help to farmers, consumers, the government, and the scientific community as will give enlightenment on the parasites prevalence rate of parasites affecting Bitter leaf (*Vernonia amygdalina*) in the study area as well as proffer solutions to the control or eradication of the parasites.

Materials and Methods

The rhizospheres of the Bitter leaf (*Vernonia amygdalina*) samples used for this study were obtained from domestic gardens in three communities in the Obio/Akpor Local Government Area, Rivers State, Nigeria. Obio/Akpor Local Government Area has the geographical coordinates of 4.8776^{o} N, 7.0283^{o} E. The sampled communities are Rukpokwu (4.9036^{o} N, 7,0015^{o} E), Ogbogoro (4.8451^{o} N, 6.9290^{o} E) and Rumuolumeni

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations,4*(1), 182-192.

(4.8115^{o} N, 6.9478^{o} E). Obio/Akpor Local Government Area plays host to some multinational oil companies as well as some government institutions. Some of the inhabitants; both natives and immigrants still practice farming in some of the rural and semirural communities. Farming in this area is however on the decline because of the gradual urbanization of the area which has led to loss of farmland. Bitter leaf (*Vernonia amygdalina*) is however found mostly in domestic gardens of the residents of the Local Government Area. Some households cultivate this plant in large quantities for home and or commercial purposes.

With gloved hands, soil samples were collected from around the roots of Bitter leaf (*Vernonia amygdalina*) in the sample locations by 08:00am each collection day, put in black polythene bags and labelled appropriately with the use of a permanent marker pen and masking tape. The dates and location of the collection were written on the bags containing each sample. All the samples collected were immediately transported to the Biology laboratory, Ignatius Ajuru University of Education Rumuolumeni Port Harcourt, for further parasitological analysis.

The modified Bearmann's technique was employed for the extraction of the nematode parasites from the rhizosphere of the Bitter leaf (*Vernonia amygdalina*) samples. About 50g of soil samples were wrapped in a serviette/tissue paper and placed on mesh/wire gauze suspended on sieve plates containing water. The set-up was allowed to make and maintain contact with the water inside the plate. It was further allowed to stand on a flat surface for 48 hours to enable the nematodes to crawl from the soil samples into the water in the plate. After 48 hours, the samples in the serviette/tissue paper were discarded and about two to three drops of 5% formalin were added to the water to anaesthetize the moving nematodes. After about 4 minutes the water was decanted and the sediments were transferred into the universal/samples bottles for onward microscopy.

Microscopy was done by placing a drop of water (sediment) containing nematodes at the centre of the slide with the use of a 3ml Pasteur pipette. This was stained with 1-2 drops of Lugol's iodine collected with another pipette, to enhance visibility. The nematode on the slide was viewed under a light microscope using the x4, x10 and x40 objectives. An identification key for nematodes was used and the population of nematodes were counted and recorded.

Data obtained from this research work was analysed using simple percentages. Results were also presented in tables and graphs. The Statistical Package for Social Sciences (SPSS) version 23 was used to compare the association between variables and a p-value less than 0.05 was considered significant.
All parasites isolated from the soil samples were recorded and the summation of all parasites identified was recorded as the overall prevalence of parasites associated with the soil samples were calculated for the overall percentage as follows:

$$\frac{\text{No soil samples positive}}{\text{Total No. of samples examined}} \times 100$$

Results

Results from this research show that out of the 30 soil samples collected (10 each) from the gardens in the three locations, 24(80%) were found to habour 3 species of plant parasitic nematodes (Table 1.). Three types of plant parasitic nematode were encountered (isolated and identified) from the three locations: (Rumuolumeni, Ogbogoro and Rukpokwu) sampled in this study. The plant parasitic nematode and their percentage occurrence are *Criconemalla* spp. 9(30%) with a total individual count of 126, *Meloidogyne* spp. 8(26.7%) with a total individual count of 64 with *Pratylenchus* spp. showing the least occurrence rate of 7(23.3%) with a total individual count of 21 (Table 2.).
Three locations from Obio/Akpor Local Government Area were sampled for this study. Incidentally, the rhizosphere from the three communities showed a very similar pattern of infection. The communities and their prevalence rates are recorded as follows: Rumuolumeni community had a parasite occurrence rate of 8(80%), Ogbogoro had a parasite occurrence rate of 8(80%), and Rukpokwu had a plant parasite prevalence rate of

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations,4*(1), 182-192.

8(80%). Chi-square statistics showed that the relationship between location and infection was not-significant ($p>0.05$) (Table 3.).

Table 1. The overall prevalence of parasites in the study area (n=30)

Total No. of soil samples examined	No. positive (% prev.)	No. negative (%)
30	24 (80)	6 (20)

Table 2. Types of plant parasitic nematodes seen in the study area (n=30)

Types of parasites	No. examined	No positive	% prevalence	Total No. of each genus
Criconemella spp.	30	9	30.0	126
Meloidogyne spp.	30	8	26.7	64
Pratylenchus spp.	30	7	23.3	21
Total	30	24	80	211

Table 3: Occurrence of plant parasitic nematodes about location (n=30)

Location	No. examined	No. positive (% prev.)	No. negative (%)	Total No. of PPN recovered
Rumuolumeni	10	8 (80)	2 (20)	92
Ogbogoro	10	8 (80)	2 (20)	67
Rukpokwu	10	8 (80)	2 (20)	52
Total	30	24 (80)	6 (20)	211

Chi-square (X^2) = 0, d.f = 2, p = 1. (Statistically non-significant)

This study revealed an 80% infection rate of all the rhizosphere of bitter leaf (*Vernonia amygdalina*) samples obtained from the three locations examined for this study and revealed the presence of three plant parasitic nematodes; *Criconemella* spp., *Meloidogyne* spp., and *Pratylenchus* spp. However, the parasite from the genus Criconemamalla was more in occurrence while *Pratylenchus* spp had the least nematode count in all the soil samples investigated. From Rumuolumeni a total count of 92 nematodes was obtained. From the Ogbogoro community, a total of 67 nematodes were obtained while from Rukpokwu nematode count from all the samples was 52. The wetness of the soil samples used for this study is recorded thus; soil samples from Rumuolumeni were wet and semi-formed, followed by soil samples from Ogbogoro while soil samples from Rukpokwu were dried and crispy. The disparity in plant parasitic nematode figures obtained from the three locations suggests that wet soil increased the number of plant parasitic nematodes, especially *Criconemella* spp.

The other two plant parasitic nematodes encountered in this research are also of economic importance to farmers and scientists. *Melodogyne* spp., are distributed throughout the world. Altogether there are 98 known *Meloidogyne spp*. which causes economic losses of about 5% throughout the world (Khanal et al., 2016). Major host ranges are cover crops, fruit trees, weeds, and ornamental and agronomic plants (Khanal et al., 2016). The symptoms include the formation of galls or knots due to the expansion of root cells. The secondary symptoms are wilting, yellowing of leaves, nutrient deficiency, and slow or stunted growth (Ralmi et al., 2016). For their Life cycle, they are migratory or sedentary endoparasitic as they enter into the root region and feed their content (Das et al., 2015). The total length of the life cycle in most of the species is about 3-4 weeks under the suitable temperature of 27-300C. Their mode of reproduction is parthenogenesis which sometimes occurs by amphimixis (Singh et al., 2013).

Conclusion

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations,4*(1), 182-192.

This study revealed that the rhizosphere Bitter leaf (*Vernonia amygdalina*) grown in the domestic gardens of the three communities; Rukpokwu, Rumuolumei and Ogbogoro all in Obio/Akpor Local Government Area, sampled for this research are heavily infected with medically important parasites which are sources of disease transmission.

Recommendation

It is recommended that adequate efforts should be put in place by the government to provide nematicides to the farmers and make them available to both commercial and peasant farmers and serious awareness of the implications of these plant parasitic nematodes should be carried out regularly in order to reduce the burden of plant parasitic nematodes as well as curtail economic losses due to these parasites.

References

Adams, A., & Motarjemi, Y. (2017). Basic Food Safety for Health Workers. H: /FichiersLY/Claudia Corazzola/Mise en page –*Basic Food Safety*.*6*, 23 – 31.

Adegbite, A. E., & Sanyaolu, E. B. (2009). Cytotoxicity testing of aqueous extract of bitter leaf (*Vernonia amygdalina* Del.) using the *Allium cepa* chromosome aberration assay. *Scientific Research and Essays*. 30;4 (11),1311-4.

Alhabbal, A. T. (2015). The Prevalence of Parasitic Contamination on Common Cold Vegetables in Alqalamoun Region. *International Journal of Pharmaceutical Sciences Review and Research*. *30*(1):94–7.

Ami, S. N., & Taher, I. I. (2013). Wheat Seed Gall Nematode *Anguina Tritici* in Duhok Province, Kurdistan Region-Iraq and its Biology. *Science Journal of University of Zakho*, *1*(2), 674-685.

Atangwho, I. J., Ebong, P. E, Eyong, E. U., Williams, I. O., Eten, M. U., & Egbung, G. E. (2009). Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. *African Journal of Biotechnology*, *8*(18).

Bauters, L., Haegeman, A., Kyndt, T., & Gheysen, G. (2014). Analysis of the transcriptome of *Hirschmanniella oryzae* to explore potential survival strategies and host–nematode interactions. *Molecular plant pathology*, *15*(4), 352-363.

Bekele, F., Tefera, T., Biresaw, G., & Tsegaye, Y. (2017). Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. *Infectious Diseases of Poverty.* 6:19.

Bertoia, M. L., Mukamal, K. J., Cahill, L. E., Hou, T., Ludwig, D. S., Mozaffarian, D., Willett, W. C., Hu, F. B., & Rimm, E. B. (2015). Changes in intake of fruits and vegetables and weight change in United States men and women followed for up to 24 years: analysis from three prospective cohort studies. *Plos Medicine. 12*(9), e1001878.

Biressaw, S. S., & Birhanu, G. (2017). Review on Nutritional and Medicinal Values of *Vernonia amygdalina* and its Uses in Human and Veterinary Medicines. *Global Veterinarian,19*(3),562-568.

Ebong, P. E., Atangwho, I. J., Eyong, E. U, & Egbung, G. E. (2008). The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss)(Neem) and *Vernonia amygdalina* (Del.) (African bitter leaf). *American Journal of Biochemistry and Biotechnology*, *4*(3):239-44.

Esteves, I., Maleita, C., & Abrantes, I. (2015). Root-lesion and root-knot nematodes parasitizing potato. *European journal of plant pathology*, *141*(2), 397-406.

Farombi, E. O., & Owoeye, O. (2011). Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International Journal of Environmental Research and Public Health. 8*(6),2533-55.

Goss, C. R. (2008). Plant parasite nematode of coffee; (1st ed.). *Springer Netherlands.* 64399.

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations,4*(1), 182-192.

Gozel, U. Ğ. U. R., Adams, B. J., Nguyen, K. B., Inserra, R. N., Giblin-Davis, R. M., & Duncan, L. W. (2006). A phylogeny of Belonolaimus populations in Florida inferred from DNA sequences. *Nematropica*, 155-172.

Guerena, M. (2006). Nematodes: alternative controls, 1-20. *ATTRA*.

Ifeoluwa, T., Oyeyemi, Akinbiyi, A., Akinlabi, B., Aderiike, Adewumi, B, Abimbola, O., Aleshinloye, Oyetunde, T., Oyeyemi. (2017). *Vernonia amygdalina*: A folkloric herb with anthelminthic properties. *Beni-Suef University Journal of Basic and Applied Sciences.*;7:43–49

Jones, M. G. K., & Fosu-Nyarko, J. (2014). Molecular biology of root lesion nematodes (*Pratylenchus spp*.) and their interaction with host plants. *Annals of applied biology*, *164*(2),163-181.

Khanal, C., Robbins, R. T., Faske, T. R., Szalanski, A. L., McGawley, E. C., & Overstreet, C. (2016). Identification and haplotype designation of *Meloidogyne spp*. of Arkansas using molecular diagnostics.

Kumar, Y. & Yadav, B. C (2020). Plant-Parasitic Nematodes: Nature's Most Successful Plant Parasite. *International journal of research and review, 7*(3),379-386.

Mandal, H. R., Katel, S., Subedi, S & Shrestha, J. (2021). Plant Parasitic Nematodes and their management in crop production: a review. *Journal of Agriculture and Natural Resources*, 4(2),327-338

Michel, J., Abd Rani, N. Z., Husain, K. (2020). A Review on the Potential Use of Medicinal Plants from Asteraceae and Lamiaceae Plant Family in Cardiovascular Diseases. *Frontiers in Pharmacology*, 11:852.

Mukhtar, T., Jabbar, A., Raja, M. U., & Javed, H. (2018). Re-emergence of wheat seed gall nematode (Anguinatritici) in Punjab, Pakistan. *Pakistan Journal of Zoology*, *50*(3).

Nwosu, S. I., Stanley, H. O, & Okerentugba, P. O. (2013). Occurrence, types and location of calcium oxalate crystals in *Vernonia amydalina* Del (Asteraceae) *Int. J. Sci. Nat.*, *4*(3),533-537

Ojimelukwe, P. C. & Amaechi, N. (2019). Composition of *Vernonia amygdalina* and its Potential Health Benefits. *International Journal of Environment, Agriculture and Biotechnology (IJEAB), 4*(6),1836.

Okeke, O. A., Udemba, N. N., Udeh, N. P., Imakwu, C. A., Nwadike, C. C., Nnatuanya, I. O., Elosiuba, N. V., Egwuagu, C. C., Afoemezie, P. I. & Obudulu, C. (2022). Parasitic Contamination of Fresh Bitter Leaf Vegetables - Onugbu (*Vernonia amygdalina*): A Case Study of Farms in Nimo, Njikoka Local Government Area, Anambra State, Nigeria. *Annual Research & Review in Biology 37*(1),43-49.

Oseni, K., & Babatunde, O. (2016). *Vernonia amygdalina*: An Underutilized Vegetable with Nutraceutical Potentials – A Review Turkish Journal of Agriculture - Food Science and Technology.*4*(9),763-76.

Ozberk, I., Yolcu, S., Yücel, A., Köten, M., & Nicol, J. M. (2011). The impact of seed gall nematode on grain yield, quality and marketing prices on durum wheat in Anatolia, Turkey. *African Journal of Agricultural Research, 6*(16), 3891-3896.

Pem, D. & Jeewon, R. (2015). Fruit and Vegetable Intake: Benefits and Progress of Nutrition Education Interventions- Narrative Review Article. *Iranian Journal of Public Health*. *44*(10),1309–1321.

Regmi, H., Nongmaithem, B. D., & Ngangbam, A. K. (2016). Biotic constraints in rice productivity with special reference to rice root nematode: *Hirschmanniella spp*. A review. *Journal of Global Agriculture and Ecology, 5*(3), 127-133.

Shah, M. M., & Mahamood, M. (2017). Introductory chapter: nematodes- a lesser known group of organisms. *Nematology- Concepts, Diagnosis and Control*, 1. 68589

Shurleff, M. C., & Averre, C. W. (2000). Diagnosing plant disease caused by plant parasitic nematodes. *The American Phytopathol Society.* 1967

Singh, S. K., Hodda, M., Ash, G. J., & Banks, N. C. (2013). Plant-parasitic nematodes as invasive species: characteristics, uncertainty and biosecurity implications. *Annals of Applied Biology, 163*(3), 323-350.

Stirling, G. R., Nicol, J., & Reay, F. (2013). Advisory services for nematode pests. *Rural Industries Research and Development Corp*.

Stirling, G. R., Stirling, A. M., Giblin-Davis, R. M., Ye, W., Porazinska, D. L., Nobbs, J. M., & Johnston, K. J. (2013). Distribution of southern sting nematode, Ibiporalolii (Nematoda: Belonolaimidae), on turfgrass in Australia and its taxonomic relationship to other belonolaimids. *Nematology*, 15(4), 401-415.

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations,4*(1), 182-192.

Subedi, S., Thapa, B., & Shrestha, J. (2020). Root-knot nematode (*Meloidogyne incognita*) and its management: a review. *Journal of Agriculture and Natural Resources*, *3*(2), 21-31.

Taha G. Ü., Ayşe, Nur, S., Onur, Ç. & Funda, P. Ç. (2018). Role of Vegetables in Human Nutrition and Disease Prevention, Vegetables - Importance of Quality Vegetables to Human Health, Md. Asaduzzaman and Toshiki Asao, Intech Open;. 77038.

Umeanaeto, P. U., Nwofor, O. F., Iwueze, M. O., Ugwuanyi, I. K., Egbuche, C. M. & Igbodika, C. M. (2016). Parasite contamination of edible vegetables sold in Onitsha Markets, *The Bioscientist*. *4*(1),21-31.

Verdejo-Lucas, S., &, McKenry, V. (2004). Management of the citrus nematode, *Tylenchulus semipenetrans*. *Journal of nematology*, *36*(4), 424.

Yu, Y. T., Liu, H. L., Zhu, A. G., Zhang, G., Zeng, L. B., & Xue, S. D. (2012). A review of root lesion nematode: Identification and plant resistance. 24052.

Das, D., Kshetrimayum, S., Devi, S., & Rai, D. (2015). A taxonomic review of Root Nematode. *International journal of current research*, *7*(12),23413-23416.

Ralmi, N. H. A. A., Khandaker, M. M., & Mat, N. (2016). Occurrence and control of root knot nematode in crops: a review. *Australian Journal of Crop Science*, *11*(12),1649.

APPENDIX 1

Occurrence of plant parasitic nematodes in relation to location (n=30)

Location	No. examined	No. positive	No. negative	**Total No. of PPN recovered**
Rumuolumeni	10	8	2	**92**
Ogbogoro	10	8	2	**67**
Rukpokwu	10	8	2	**52**
Total	30	24	6	**211**

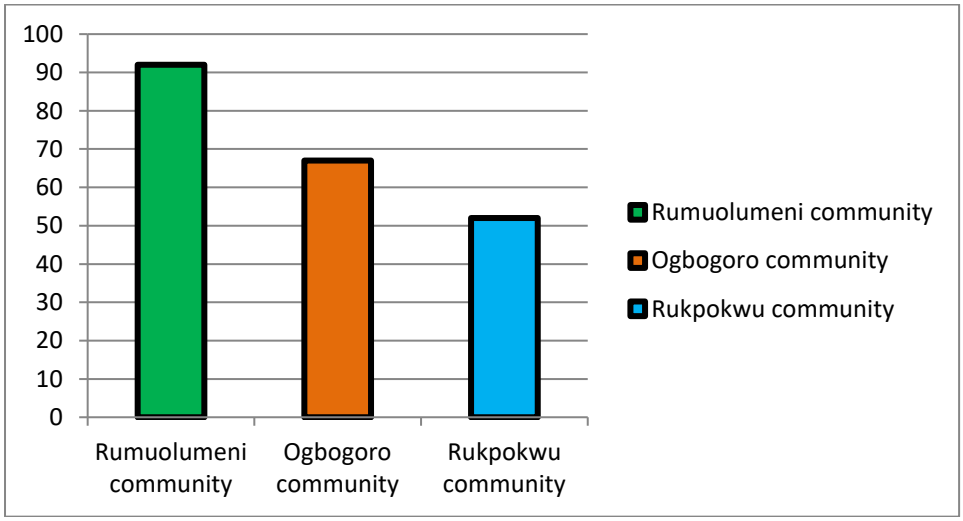

Fig. 1. Graphical representation of parasitic infection in the different communities

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations*,*4*(1), 182-192.

APPENDIX II
PICTORIAL REPRESENTATION OF PARASITES SEEN IN THE LABORATORY AND THE PRACTICAL SESSIONS

Plate 1. Photo of *Criconememalla* spp. (with spines) as seen in the laboratory x40 objective lense

Plate 2. Central view of *Criconememalla* spp. as seen in the laboratory x40

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations*,*4*(1), 182-192.

Plate 3. Posterior end of *Criconememalla* spp. as seen in the laboratory x40

Plate 4. Photo of *Criconememalla* spp. As seen in the laboratory x10 objective lense

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations*,*4*(1), 182-192.

Plate 5. Experimental set up for nematode parasite extraction in the biology laboratory: the modified Baermenn's method

Cite this article as:
Elele, K., Anwuri, N., & Ogbuku, C.P. (2023). Parasitic analysis of the rhizosphere around the roots of bitter leaf (*vernonia amygdalina*) grown in Obio/Akpor Local Government Area Rivers State Nigeria. *FNAS Journal of Scientific Innovations*,*4*(1), 182-192.