*International Journal of Innovative Agriculture & Biology Research 14(1):38-51, Jan.-Mar., 2026*

 **www.seahipublications.org** **ISSN: 2354- 2934**

**doi:10.5281/zenodo.18754618**

# Comparative Studies on the Nematicidal Efficacy of Ethanol Leaf Extract of *Azadirachta indica* and *Vernonia amaegdalina* on Root-Knot Nematodes of Cowpea in Sokoto State

**[1]Liman B, [2] Ukatu V.E., [1]U.Tambari, &[1] Marwan .A**

**[1]Department of Biology, Shehu Shagari College of Education, Sokoto, Nigeria**
**[2]Department of Animal and Environmental Biology, Afusta, Kebbi State, Nigeria**
**Correspondent Author: limanbashiru.bl@gmail.com +2348065717647**

**ABSTRACT**

Root-knot nematode infection is one of the greatest menace affecting the growth and yield of many crops not only in Nigeria but globally. The present study aimed at conducting a greenhouse experiment to compare the efficacy of Ethanol leaf extract of *Azadirachta indica* and *Vernonia amaegdalina* for the control of root-knot nematodes of cowpea. A questionnaire method was used to obtain information from the local farmers in Sokoto State on the type of plants they use to treat suspected cases of root-knot infection. A total of 200 questionnaires were distributed. A total of nine plants were identified out of which 2 plants were selected for the experiment, based on the frequency of appearance of the plants in the responses. Thirty plastic pots mouth open of 15cm were obtained and filled with autoclaved soil. One week old cowpea seedlings raised separately was uprooted and transferred in to the pots and inoculated with 1000 juveniles of nematodes except the first group that was left as control. Ethanol extract of leaves of *Azadirachta indica* and *Vernonia amaegdalina* was measured at 10ml and applied in to the inoculated seedlings at different concentration (100mg/ml, 200mg/ml, 300mg/ml & 400mg/ml) except the second group which was inoculated and left untreated to serve as another control. Four weeks after the transplant, growth was measured based on some parameters ie plant height, root length, extent of galling as well as total number of nematodes. *Azadirachta indica* shows high efficacy as nematicides compared to the Vernonia leaf extracts especially at high concentration (400mg/ml). It is therefore recommended for farmers to use them as an alternative control for nematodes infection.

**Keywords:** *Azadirachta indica, Vernonia,* nematicides, Root-knot nematode

## INTRODUCTION

Agricultural Produce means all produce and commodities, whether processed or unprocessed, of agriculture, horticulture, apiculture, sericulture, livestock and products of livestock, fleeces (raw wool) and skins of animals, forest produce and fisheries. It may also include two or more of such products (Wang *et al*., 2019).Civilization started with agriculture which, to this day, remains very important and plays a significant role in our lives. While its significance may be even more pronounced in some countries than others, the reality is that every country depends on agriculture to sustain itself in one way or another (Folawiyo, 2018). For decades, it's been associated with the production of food crops. However, the importance of agriculture goes above and beyond farming. It's evolved into forestry, fruit cultivation, beekeeping, arbitrary, mushroom, dairy, etc. Today, the processing, distribution, and marketing of crops and livestock products are all acknowledged as a part of agriculture.

Agriculture plays an essential role in sustaining and driving the economy. It's the backbone of everything that drives us. In addition to providing food and other raw materials, it also provides employment opportunities. Safe to say the importance of agriculture cannot be overstated (Mark, 2021)

The nematode parasites of plants (the causal organism of ear cockle of wheat) were reported as early as 1743. However, the importance of these tiny organisms in the scheme of human welfare is of much later realization. There had been stray cases when these organisms had been found to cause significant damage in the nineteenth century (such as the sugar beet cyst nematode) but it was only after the second world war (1945), when some easy to apply and relatively cheap nematicides were discovered, that their significance as widely occurring destructive plant parasites was demonstrated.

Many crops including cowpea are susceptible to nematodes attack, for instance an evaluated yield loss of bean cultivar was found out that 45 to 63% yield loss was due to nematodes. Several workers like Taylor 2019,Ritter 2022, and Adamova 2015, reported losses of up to 50% of the total gross production of vegetable and 2% of the fruits due to root nematodes in southern Europe and Mediterranean.

On a global basis, out of the approximately 34% crop losses annually caused by crop enemies like fungi, bacteria, nematodes, viruses, insects, and weeds, nematodes alone are responsible for losses of about 11% (*Broodier et al.,* 2022). According to Sasser and Freckman (2010), the annual loss of crops due to nematodes is about 12.3%, more in the developing countries like Nigeria than in the developed countries of Europe and America. Losses in potato, cowpea, eggplant, Okra and pepper (chilli) are 12.2, 20.6, 16.9, 20.4, and 12.2%, respectively (Olowe, 2021). Loss of yield in cowpea is estimated to be 60 to70% (Odihirifi, 2018). Even in a developed country like the USA about 7.2% of the annual crop value was lost to nematode attacks during the mid-sixties of the last century, in England, the potato cyst nematode caused annual loss of about 20 million dollars(*Sasser et al,. 2017*). The root knot nematodes annually destroy 29 to 90 % of vegetable crops (Alose, 2023). In Nigeria, reduction in yield of cowpea due to root-knot is reported to range from 26.5 to 43. 3% (Shaukat *et al*, 2019). In legume-crops, Suatmadji (2019) have estimated a loss of 13.7% in chickpea due to root- knot and 13.2% in pigeon pea due to root-knot, cyst nematode and *Rotylenchulus reniformis*. Citrus decline, pepper yellows, molya disease of wheat and barley (cereal cyst nematode), and ear cockle of wheat are some of the examples of the damage caused by plant parasitic nematodes. There is hardly any horticultural crop that is not attacked by nematodes, even some grain crops suffer heavily from their attack (Suatmadji 2019)

Symptoms of Nematode infection are mostly non-specific. Poor growth of the plant, stunting, patchiness of the crop and discoloured foliage are such abnormalities that can be attributed to many other causes such as attack of fungi, bacteria and viruses as well as soil factors including nutritional disorders and unsuitable soil physical conditions. While some nematodes are primary pathogens, others by feeding on the host plant, may allow entry of other pathogens or secondary invaders which cause the damage and visible symptoms. In many diseases, there is clear association between nematodes and other pathogens (Wallace, 2018).

The above ground symptoms include reduced growth, discolouration of foliage, and distortion of plant parts. Stunting and slow growth are common symptoms. Due to non-random distribution of nematodes in the field, such plants appear in patches and generally pronounced stunting occurs when the nematode population is high. Leaf chlorosis often accompanies stunted growth of plants. The yellowing indicates nutritional deficiency caused by root destruction and immobility of nutrient from the root to the shoot. The discoloration may range from light yellow to deep red, purple or even black. Distortion of aerial parts is generally caused by stem and leaf feeding nematodes.

These distortions include swellings on leaves and stems, twisting of leaves, and replacement of grains with cockles. The root parasites induce conspicuous below ground symptoms which include reduced root system, root proliferation, root galls and cysts on roots. Depletion of the root system is the most common below ground symptom of nematode attack. In some cases root growth inhibition is the only symptom. The roots may show lesions in addition to growth inhibition. Some species of nematodes do not cause general decay of roots. The injury to roots induces the plant to grow more roots in clusters, especially behind the damaged portion. Galling of roots is the most characteristic symptom of attack of root-knot nematodes. However, many other nematodes such as *Xiphinema* and *Globodera* also cause swelling of underground parts of their host. In the attack of cyst- nematodes, presence of white to brown cysts projecting on the root surface is characteristic symptom.

Damage to crops by nematodes is related to the population of the nematodes attacking the crops. Controlling such pests mean lessening the number until yield loss is economically acceptable, (Adesiyan *et al.*, 2010). Maintenance to acceptable limit, might require outright killing of the nematodes using chemical, or may be achieved indirectly by adding soil amendments that encourage the growth of the host plant and antagonists that kill the nematodes. A number of control practices have been used over time. These control methods includes chemical control, use of resistant varieties, crop rotation, biological control, and recently the use of plant extracts and soil amendments.

Once Endoparasitic species have penetrated a root, control with chemicals is more difficult as nematicidal compounds have to be non-phytotoxic and preferably systemic. Nematicides that can be safely applied to growing plants and is translocated to the roots in sufficiently large amounts to kill endoparasitic or ectoparasitic nematodes have not been discovered. Oxamyl, a systemic compound that is translocated basipetally, is the only commercial product that is used as a foliar treatment, but its use as a liquid formulation is restricted in many countries for toxicological reasons (Hague and Gowen, 2017).

There are several nematicides that can be used effectively for nematode pests of annual crops (Van Berkum and Hoekstra, 2019), but there appears to be little prospect for management of nematodes in many susceptible, perennial crops without repeated application of nematicides. Only in certain cases will such treatments be justified economically. Nematicides are highly toxic compounds that have very low LD50 values. This is particularly important for operators of application machinery and people at risk from exposure to the chemicals during their application.

The liquid formulations of some of the non-fumigant nematicides are emulsifiable concentrates. Their use should therefore be restricted to skilled operators who take adequate safety precautions. This may not always be the case where basic levels of education are poor or where operators cannot read the instructions on the labels of the products. The application of nematicides to crops too near to harvest is another risk which pesticide residue monitoring may not be sufficiently well coordinated to prevent poisoning.

The availability and quality of food and the ability of the parasites to secure and use it are dominant actors in population growth (Powel, 2021).The status of host plant and the factors influencing it are therefore major determinant in the dynamics of established population and have received much attention. Some plant varieties show resistance to invasion by nematodes. Such resistance is brought about by the development of hypersensitivity reaction as a result of nematode's infestation. As no giant cells are formed in a resistant variety, nematodes are surrounded by dead cells, thus depriving them of nutrients, resulting in their death. It was found that syncytial formation was inhibited in resistant varieties of manoharsali and Baharsa. Odihirifi (2018) found TVU 857 cowpea very resistant to both *M.incognita* and M. *Javanica.* In the same vein Olowe (2021) found that a cultivar of cowpea 64298 consistently showed some level of resistance to races 1, 3, and 4 of *M. incognita* and resistance to *M. Areanarea* and viral infection.

Cowpea cultivar ACC 67010 also showed resistance to *M. areanarea in* general, resistance to nematode infestation may be developed through breeding. This is through the incorporation of resistant genes in a susceptible variety through breeding programme. By careful screening of such hybrids, resistant lines may be related to the presence of nematicidal substances in the plant (Adesiyan *et al.*, 2010).

Crop rotation here implies rotating a susceptible crop with the non-susceptible ones. The chemical control is becoming increasingly uneconomical because of the high cost of chemicals; expensive nature of their applications and their undesirable side effects, hence the need for alternative control measures. Planting crops in special sequence (such as Rotation) has been an old, effective and widely used land management practice for reducing nematode population in the soil (Mahmud and Siddiqui, 2021). Crop rotation is a successful farming practice, not only because of its beneficial soil building effect, but also because nematodes populations can be regulated (Good *et al.*, 2018).

In Nigeria, root-crops, cereals, legumes and vegetables are grown in mixture on most traditional agricultural fields. The root-knot nematodes infest these groups individually, but with limited economic losses on cereals (Egunjobi, 2015). It was further reported that in intercropping system, maize confers significant protection to Cowpea against root-knot nematodes.

The only problem with crop rotation is that, one designed to reduce a given nematode species, often lead to the increase in the population of other species to damaging levels (Brodies *et al.*, 2017).

Another problem is that the immune crop may not always be as profitable as the susceptible; hence farmers unwillingness to adopt the method. It should therefore be noted that, for crop rotation to be economically acceptable to the farmer, it must not only increase and protect yields, but the crops in rotation must be of economic value to the user.

Baker and Look (2018) defined biological control as the reduction of inoculums density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organism, accompanied naturally or through manipulation of the environment of the host or its antagonist by mass introduction of one or more antagonists. Biological Control in this case, therefore implies the use of any live material (Plant or animal) for the control of nematodes. Several workers have used fungi, bacteria and other nematodes to control some plant parasitic nematodes

A number of plants are thought to have contained extracts with some nematicidal properties. The nematicidal activiti.es of plant has been investigated by several workers. These substances have been found to reduce a popular level of some nematodes species. Invitro test of water extracts leaves stems and bark of *tylencus spp* and that of *Anguina spp* indicated 50-70% mortality of nematodes at 4mg/mi dilution after 48hrs of treatments. The water extracts of neem oil cake and leaves renders the root of susceptible plants highly unfavourable to *Meloidogyne* and similarly the use of such extracts have been found to render the roots unfavourable for nematodes hence causing poor penetration and later retardation in the biological activity such as feeding and reproduction of root-knot nematodes.

Gowen and Ahmed (2018) listed a number of plant species whose extract possess the ability to control various nematodes species. These included Ageratum *conyoides* acting on *Meloidogyne incognita and M.javanica; Alllantus excels* acting on *M. incognita and M.javanica; Brassica nigra* acting on *Tylenchorhynchus brassica* where the extracts are absorbed by the roots.

**Statement of the Problem**

Nematodes are known to cause a lot of damage to many plants including cowpea, Cowpea, Carrots etc.Rarely any crop is free from their attack, yet they escape notice because of their microscopic size and protective position (Southey, 2016). Many control measures such as chemical method, crop rotation, etc. have been put in place with little or no success. Although some are quite effective, their side effects by far outweigh the success. The widespread use of synthetic pesticides has significant draw back including development of strain resistant to insecticides, increased cost, handling hazards, concern about threat to human health, animal and environmental components. Repeated use of synthetic chemicals for pest and vector control has disrupted natural biological control system; it has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern. It is therefore pertinent to find a way of reducing the yield loss incurred by these pests using the safest way of disease management, in order to boost world food production. Nowadays there is growing awareness on the hazards of using chemicals in plant disease control, both on the soil nutrients and the individual himself. These resulted in banning the use of some chemical such as the D.D.T. Interest is therefore shifting towards the use of plant extracts in disease management. This therefore necessitated this research to be conducted with the aim of using natural plant materials in the control of the root-knot nematodes (*Meloidogyne*) of cowpea and cowpea.

**Justification**

This research is important to farmers and to the society because it provide an alternative method of nematode control which has been a great menace and an obstacle in the yield and production of many important crops including Cowpea. Economically, it also provides a cheaper and safer method of plant disease management, which complements other nematode control methods. Chemical fungicides and pesticides are used for effective control of phytopathogens. However, the deleterious effects of these chemicals on human health and environment strongly demand the search for an alternative eco-friendly approach for Pathogen control. Use of plant extracts provides a safer, Biocompatible and nontoxic methods of control of plant parasites and has received great attention as a Biopesticides.It enhances promotion of plant growth and disease control and thus can improve the Bio-availability, stability, of fungicides or pesticides. It is important scientifically for other researchers in the field to be able to make further enquiry in plant disease management and specifically in the control of root-knot nematodes.

**Aim and Objectives of the Work**

The aim of this work is to compare the nematicidal potentials of Ethanol extracts of *Azadirachta indica* and *Vernonia amaegdalina* on root-knot nematodes of cowpea. The specific objectives are to:-

i. Collect, identify and select the *Azadirachta indica and Vernonia amaegdalina* in sokoto
ii. determines the phytochemical composition of the tested plants
iii. determine the quantitative analysis of the tested plants
iv determines the effects of the extracts on the plant height, root length, number of galls as well as nematodes population of the cowpea inoculated with root-knot nematodes.

## METHODOLOGY

**Study Area**

The study was carried out in Sokoto South and north local government area of Sokoto State which is located in the extreme northwest of Nigeria and has a land mass area of 28,232 square kilometers with the coordinate of $13^0 0^0$ 21.14288" N and $5^0$ 14" 51.1872" E .It shared borders with Niger republic in the east, Kebbi state to the west and Zamfara state to the south. Sokoto is the state capital and the state has an estimate population of about 4.2 million according to (NPC, 2006)

The majority of the population are Hausa /Fulani although there are also a significant population of Yoruba ,Igbo, Kabawa, Zamfarawa, Igala, Nufawa and host of other tribes like Gwarawa, Dakkarawa, and Nufawa also residing in the state( Arshad and Odeh,2014)

The occupation of the residents includes civil service, dying, weaving, crafting, artisan and much other hand work. However Sokoto people are highly dependent on agricultural activities such as farming and rearing of animals during rainy and dry season, the people are highly dependent on both subsistence and commercial farming. During the dry season, the people of sokoto also practice irrigation farming that allows the cultivation of cowpea, carrot, cabbage onions, ginger and many other vegetables (Ismail and Oke, 2012)

Sokoto state is located in the dry Sahel region with an average temperature of $28.3^0$ ($882.0^0$F).Although the area is generally very hot, the dryness helps keep the highest temperature below $40^0$ C for the majority of the year. The warmest months are February to April when daytime temperatures can exceeds $45^0$ ($113.0^0$ F) (Kasim and Usman, 2021).The harmattan winds blows Sahara dust over the region and dominates the climate from June to October which is the rainy season, this wind reduces sunlight which lowers temperatures and causes dust to accumulate in homes (Jibrillah *et al.*, 2019).

**Administration of Questionnaire**

A questionnaire was administered to the local farmers within the study area in order to ascertain the types of plants they use to treat symptoms of nematodes infection. The questionnaire comprised of six sections namely: Age of farmer, Local government area, name of senatorial zone, name of collecting centre, type of crops raised, name of plants and or parts used when symptoms of nematodes infection is suspected. The responses gotten were used as the basis for the selection of the plants used for the experiment.

**Collection and Identification of Plants**

The collection and identification of plant material that are commonly used as nematicides was done with the help of farmers in the study area. The study area was grouped in to two zones (A, and B) according to the local government in the study area.in order to collect information on the type of plants they use as nematicides in the area. The plant name which appeared more frequent in each local government was chosen as one of the experimental plant in the study area Thus, two (2) plants *Azadirachta indica* and *Vernonia amaegdalina* were selected as candidate plants in the study area . The plants collected were uthenticated at the department of Biological science, Usmanu Danfodiyo University Sokoto, where they were identified by a taxonomist and a voucher number was assigned to them as UDUH/ANS/0985 and UDUH/ANS/0986 for, *A.indica* and *V. amaegdalina* respectively.

**Collection of Inoculums.**

Soil samples were collected from an infested cowpea farm in kwalkwalawa area along the Usmanu Danfodiyo University main campus where villagers raise their crops. Infestation was confirmed by looking at the emergence of galls within the root of the plants (Abubakar, 1999). Similarly some samples were collected in an infested farm behind bursary area of the main campus, by digging around the rhizosphere of the cowpea, 3cm below the surface of the soil using a shovel. All the samples were then placed in a polythene bags and taken to the laboratory and kept under room temperature until it is due for isolation.

**Isolation of Nematodes from the Soil Samples**

For isolation, Cobb decantation and sieving technique was employed. In this method, the soil sample was poured into a bucket half-filled with water. The mixture of soil and water was then stirred gently to remove any lump and allowed to settle for about 30 seconds. The supernatant liquid which contain most of the nematodes was decanted and poured through series of eight inches diameter sieves of varying mesh sizes (40, 80 and 120cm) some of the nematodes were trapped in the sieves. The suspension was collected in a basin and poured again for the second time over the sieves for maximum collection of nematodes. The residue from each sieve was collected, which contain the Nematodes required for the inoculation (Abubakar, 1999).

**Extraction of Plants Extracts**

The leaves of the plants chosen for this experiment obtained and identified were shade-dried; the dried parts were ground using pestle and mortar and sieve to obtain a fine powder.200g of the dried fine powder was measured and placed in 1 liter conical flask and 500ml of Ethanol was added. It was manually shaken vigorously and allowed to stand for 48 hours (2 days), it was then shaken for about 20 minutes and filtered using size 1 whatman filter paper to obtain an aqueous extract of the experimental plant. The volume was noted and placed in an electric drier and evaporated slowly at 45$^{o}$C as described by Muyibi *et al*., (2000).

**Determination of Nematicidal Effect of *Azadirachta indica and Vernonia Amaegdalina* on Cowpea inoculated with root-knot nematodes**

Extracts of leaves of the plants obtained was tested for its effects on root-knot nematodes. The extract of leaves obtained by the method earlier described was used. 30 plastic pots of 15cm diameter were obtained for the plant material to be tested. The pots were divided in to six group of five. Each pot was filled with 1kg of autoclaved soil. Each pot was inoculated with 1000 juveniles nematodes earlier isolated from the soil sample except the 1$^{st}$ group of five which serve as control. One week old seedlings of cowpea raised separately in the autoclaved soil were uprooted and transferred into the pots earlier inoculated with nematodes. Ten (10mls) of extracts of *Azadirachta indica and Vernonia amaegdalina was* measured and applied to each of the pots earlier inoculated with nematodes using the various concentrations obtained, except the first control group and second group of five that were inoculated and left untreated to serve as another control (Southey, 2016). The cowpea was watered regularly and was obtained from Sokoto central market.

Four weeks after the transplant, one cowpea plant was removed from each pot and growth was observed in terms of plant height, root length, extent of galling as well as final nematodes population. The extent of galling was established on a scale of 0- *5* (Sasser et al., 1984) as follows; where 0= no galling; 1 = 1-10 galls; 2=11-20 galls; 3=21-30 galls 4=31-100 galls; and 5= more than 100 galls per root system. The galling was identified by removing the plant and observing their emergence.

**Data Analysis**

The data obtained was subjected to analysis of variance (two ways repeated measures Anova) and Duncan multiple range tests was used to test the treatments for significance at 5% level of significance.

**RESULTS**

**Table 1: Phytochemical Constituents of the Ethanol Extracts of *Azadirachta indica***

| Phytochemical Constituents | Inferences |
|---|---|
| Alkaloids | ++ |
| Flavonoids | + |
| Tannins | + |
| Phenol | + |
| Saponins | + |
| Steriods | + |
| Glycosides | - |

**Table 2: Phytochemical Constituents of the Ethanol Extracts of *Vernonia amaegdalina***

| Phytochemical Constituents | Inferences |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Tannins | + |
| Phenol | - |
| Saponins | + |
| Steriods | + |
| Glycosides | - |

Key: + = trace, ++ = moderate +++ = high **-** = not detected

**Table 3: Quantitative Analysis of Constituents of Experimental Plants**

| Constituents | Azadirachta indica | Vernonia amaegdalina | Average | Inference |
|---|---|---|---|---|
| Alkaloids | 11.4267 | 5.2767 | 11.0400 | 2nd |
| Flavonoids | 12.8467 | 13.7300 | 13.7022 | 1st |
| Tannins | 9.0233 | 3.4867 | 9.6989 | 3rd |
| Saponin | 2.5900 | 2.2967 | 3.4244 | 4th |
| **Average** | **8.9717** | **6.1975** | | |

Table 3 presents a summary descriptive statistics of mean constituents of the experimental plants. The analysis of Alkaloids constituents of the two (2) experimental plants revealed that *Azadirachta indica* (Mean = 11.4267) has high Alkaloids content compared to *Vernonia amaegdalina* (Mean = 5.2767), while the analysis of Flavonoids constituents revealed *Azadirachta indica* (Mean = 12.8467) has the least flavonoids constituents as compared to *Vernonia amaegdalina* (Mean = 13.7300) However, the analysis of Tannins constituents showed that *Azadirachta indica* (Mean = 9.0233) that has the highest Tannins constituents as compared to *Vernonia amaegdalina* (Mean = 3.4867). In the same vein the results of Saponin constituents analysis showed that *Azadirachta indica* (Mean = 2.5900) has the highest Saponin constituents as compared to Vernonia *amaegdalina* (Mean = 2.2967). Therefore based on the analysis Azadirachta indica has the highest average mean constituents = (8.97) as compared to the V. amaegdalina (6.19) and which implies that it is the most active plant in controlling the root-knot nematodes because of its nematicidal properties.

**Table 4: Comparison of Heights of Cowpea Plants Inoculated with Nematodes and Treated with Ethanol Extract of *Azadirachta indica* and *Vernonia amaegdalina* at Different Concentrations**

| Concentration (mg/ml) | *Azadirachta indica* Mean±SEM | F | *p*-Value | *Vernonia amaegdalina* Mean±SEM | F | *p*-Value |
|---|---|---|---|---|---|---|
| Un-inoculated | 27.57±0.07 | 427.767 | 0.001 | 26.63±0.32 | 361.107 | 0.001 |
| Inoculated Untreated | 17.35±0.03 | | | 17.10±0.10 | | |
| 100 | 21.00±0.06 | | | 21.00±0.12 | | |
| 200 | 22.55±0.42 | | | 21.85±0.03 | | |
| 300 | 25.03±0.03 | | | 24.32±0.26 | | |
| 400 | 26.70±0.13 | | | 25.42±0.13 | | |
| **Total** | **23.37±0.85** | | | **22.72±0.77** | | |

Table 4 showed a summary of a Two-way repeated measures ANOVA conducted to compare the heights of cowpea plant inoculated with nematodes and treated with Ethanol extract of *Azadirachta indica* and *Vernonia amaegdalina plants* at different concentrations. The results revealed a significant improvement in the height of cowpea plant treated with ethanol extract of *Azadirachta indica* ($F(5, 12) = 427.767$, $p = 0.001$) and *Vernonia amaegdalina* ($F(5, 12) = 361.107$, $p = 0.001$) as compared to the controlled untreated cowpea plant at all levels of concentration. The mean heights of the un-inoculated cowpea plant are 27.57cm and 26.63cm respectively, indicted an optimal growth in the absence of nematodes. However, the inoculated and untreated control in which the cowpea plant was exposed to nematodes infection, exhibited a significantly lower mean heights of 17.35cm and 17.10cm respectively as compared to the un-inoculated control, demonstrating the detrimental effect of nematode infection on the cowpea plant.

In addition, the results revealed that application of different concentrations of Ethanol extract of *Azadirachta Indica* and *Vernonia Amaegdalina* plants leaves resulted in varying degrees of growth recovery from the nematodes infected cowpea plant, resulting in an improved cowpea plant height. For instance, at lower concentration (100-300 mg/ml) there was a positive effect with an increasing concentration, generally leading to an increased cowpea plant height. However, at the highest concentration (400mg/ml), the highest and most significant improvement in the cowpea plant heights of 26.70cm and 25.43cm respectively were recorded, which is almost approaching the height of un-inoculated control. Nonetheless, a *p*-value of $0.001 < 0.05$ for the treatment groups indicated that there was statistically significant difference in the height of treated cowpea plant between the treatment/concentration groups.

**Table 5: Comparison of Root length of Cowpea Plant Inoculated with Nematodes and Treated with ethanol Extract of *Azadirachta indica* and *Vernonia amaegdalina* plants at Different Concentrations**

| Ethanol Extract | *Azadirachta Indica* | | | *Vernonia amaegdalina* | | |
|---|---|---|---|---|---|---|
| **Treatment (mg/ml)** | **Mean±SEM** | **F** | ***p*-Value** | **Mean±SEM** | **F** | ***p*-Value** |
| Un-inoculated | 8.03±0.03 | 991.760 | 0.001 | 7.85±0.03 | 735.600 | 0.001 |
| Inoculated Untreated | 5.75±0.029 | | | 5.37±0.03 | | |
| 100mg/ml | 6.07±0.03 | | | 5.78±0.017 | | |
| 200mg/ml | 6.27±0.03 | | | 5.92±0.017 | | |
| 300mg/ml | 6.48±0.02 | | | 6.10±0.06 | | |
| 400mg/ml | 7.83±0.03 | | | 6.48±0.017 | | |
| **Total** | 6.74±0.21 | | | 6.25±0.19 | | |

Table 5 showed a summary of a Two-ways repeated measures ANOVA conducted to compare the roots lengths of cowpea plant inoculated with nematodes and treated with ethanol extract of *Azadirachta indica* and *Vernonia amaegdalina plants* at different concentrations. The results revealed a significant improvement in the root lengths of the cowpea plant treated with ethanol extract of *Azadirachta indica* F (5, 12) = 991.760, p = 0.001) and *Vernonia amaegdalina* (F(5, 12) = 735.600, p = 0.001) as compared to the controlled untreated cowpea plant at all levels of concentration. The mean roots length of the un-inoculated cowpea plant of 8.03cm and 7.85cm respectively both indicated an optimal growth in the absence of nematodes. However, the inoculated and untreated control in which the cowpea plant was exposed to nematodes infection, exhibited significant lower mean roots length of 5.75cm and 5.37cm of the cowpea plant respectively as compared to the un-inoculated control, demonstrating the detrimental effect of nematode infection on the plant.

In addition, the results also revealed that application of different concentrations of Ethanol extract of *Azadirachta indica* and *Vernonia amaegdalina* plants resulted in varying degrees of growth recovery from the nematodes infected cowpea plant, resulting in an improved roots length. For instance, at lower concentration (100-300 mg/ml) there was a positive effect with an increasing concentration, generally leading to an increased roots length of the infected cowpea plant. At the highest concentration (400mg/ml), the highest and most significant improvement in the roots length of 7.83 cm and 6.48cm respectively were recorded which were almost approaching the roots length of un-inoculated control. Nonetheless, a *p*-value of 0.001 < 0.05 for the treatment groups indicated that there was statistically significant difference in root lengths of treated cowpea plant between the treatment/concentration groups.

**Table 6: Comparison of Number of Galls of Cowpea Plant Inoculated with Nematodes and Treated with Extract of *Azadirachta indica* and *Vernonia amaegdalina* at Different Concentrations**

| | *Azadirachta indica* | | | *Vernonia amaegdalina* | | |
|---|---|---|---|---|---|---|
| **Treatment (mg/ml)** | **Mean±SEM** | **F** | ***p*-Value** | **Mean±SEM** | **F** | ***p*-Value** |
| Un-inoculated | 0.00±0.00 | 125.463 | 0.001 | 0.00±0.00 | 19.826 | 0.001 |
| Inoculated Untreated | 13.33±1.20 | | | 14.00±1.53 | | |
| 100mg/ml | 12.33±0.33 | | | 12.67±0.67 | | |
| 200mg/ml | 11.33±0.33 | | | 8.33±1.86 | | |
| 300mg/ml | 8.67±0.33 | | | 6.00±2.00 | | |
| 400mg/ml | 0.00±0.00 | | | 1.00±0.00 | | |
| **Total** | 7.61±1.36 | | | 7.00±1.36 | | |

Table 6 showed a summary of a Two-ways repeated measures ANOVA conducted to compare the number of galls of cowpea plant inoculated with nematodes and treated with Ethanol extract of *Azadirachta indica* and *Vernonia amaegdalina plants* at different concentrations. The results revealed a significant decrease in the number of galls on the cowpea plant treated with Ethanol extract of *Azadirachta indica* ($F(5, 12) = 125.463$, $p = 0.001$) and *Vernonia amaegdalina* ($F(5, 12) = 19.826$, $p = 0.001$) as compared to the controlled untreated cowpea plant at all levels of concentration. The mean number of galls of the un-inoculated cowpea plant was 0.00 (absent) for both treatments, indicating absence of galls and optimal absence of nematodes. However, the inoculated and untreated control in which the cowpea plant was exposed to nematodes infection, exhibited significant higher mean number of galls of 13.33 and 14.00 respectively as compared to the complete absence of galls in the un-inoculated control, demonstrating the detrimental effect of nematode infection on the plants.

In addition, the results also revealed that application of different concentrations of Ethanol extract of *A.indica* and *V. amaegdalina* plants resulted in varying degrees of reduction in the number of galls and nematodes population present in the nematodes infected cowpea plant, resulting in a decrease in the number of galls present in the cowpea plant. For instance at lower concentration (100-300 mg/ml) there was a negative effect with an increasing concentration, generally leading to a decreased number of galls in the infected cowpea plant. At the highest concentration (400mg/ml), the highest and most significant decrease in the number galls of 0.00 was recorded for treatment with *A. indica* which was equal to the number of galls for the un-inoculated control and 1.00 gall was recorded for treatment with *Vernonia amaegdalina* which was almost approaching the 0.00 number of galls of un-inoculated control. Nonetheless, a *p*-value of $0.001 < 0.05$ for the treatment groups indicated that there was statistically significant difference in the number of galls present in the treated cowpea plant between the treatment groups.

**Table 7: Comparison of Final Nematodes Population of Cowpea Plant Inoculated with Nematodes and Treated with Ethanol Extract of *Azadirachta indica* and *Vernonia amaegdalina* at Different Concentrations**

| | ***Azadirachta indica*** | | | ***Vernonia amaegdalina*** | | |
|---|---|---|---|---|---|---|
| **Treatment (mg/ml)** | **Mean±SEM** | **F** | ***p*-Value** | **Mean±SEM** | **F** | ***p*-Value** |
| Un-inoculated | 0.00±0.00 | 2997.433 | 0.001 | 0.00±0.00 | 6010.755 | 0.001 |
| Inoculated Untreated | 528.33±6.01 | | | 546.67±3.33 | | |
| 100mg/ml | 270.00±2.89 | | | 275.00±2.89 | | |
| 200mg/ml | 242.67±1.45 | | | 256.67±1.76 | | |
| 300mg/ml | 236.67±3.28 | | | 240.00±1.15 | | |
| 400mg/ml | 157.33±1.45 | | | 174.67±2.73 | | |
| **Total** | **239.17±38.17** | | | **248.83±39.23** | | |

Table 7 showed a summary of Two-ways repeated measures ANOVA conducted to compare the number of final nematodes population of the cowpea plant inoculated with nematodes and treated with Ethanol extract of *Azadirachta indica* and *Vernonia amaegdalina plants* at different concentrations. The results revealed a significant decrease in the number of final nematodes population for the treatment with Ethanol extract of *Azadirachta indica* $F (5, 12) = 2997.433$, $p = 0.001$) and *Vernonia amaegdalina* ($F(5, 12) = 6010.755$, $p = 0.001$) as compared to the controlled untreated cowpea plant at all levels of concentration. The mean nematodes population of the un-inoculated cowpea plant was 0.00 for both treatments, indicating complete absence of nematodes. However, the inoculated and untreated control in which the cowpea plant were exposed to nematodes infection, exhibited the highest mean number of nematodes population of 528.33 and 546.67 respectively as compared to the complete absence of nematodes in the un-inoculated control, demonstrating the detrimental effect of nematode infection on the cowpea plant.

In addition, the results also revealed that application of different concentrations of Ethanol extract of *Azadirachta indica* and *Vernonia amaegdalina* plants, resulted in varying degrees of reduction of the number of nematodes population in the nematodes infected cowpea plant, resulting in a decrease in the number of final nematodes population present in the treated cowpea plant. For instance at lower concentration (100-300 mg/ml) there was a negative effect with an increasing concentration, generally leading to decreased number of nematodes population in the cowpea plant. At the highest concentration (400mg/ml), the highest and most significant decrease in the number final nematodes population in the cowpea of 157.33 and 174.67 respectively were recorded which are getting approaching the 0.00 number of nematodes population of un-inoculated control. Nonetheless, a $p$-value of $0.001 < 0.05$ for the treatment groups indicated that there was statistically significant difference in the number of final nematodes population between the treatment/concentration groups.

## DISCUSSION

Root-knot Nematodes is one of the major problems affecting many crops not only in Nigeria but globally (Whitehead,2019).There is rarely any crop that escape their attacks but yet escape notice because of their microscopic size and protective position within the soil (Southey,2016). Recently there is a growing interest in using plants constituents for the control of many plant diseases.

The reduction in the number of nematodes recorded in the various cowpea plants inoculated with the root-knot nematodes and treated with the different concentration of Ethanol extracts of *Azadirachta indica* and *Vernonia amaegdalina* may be accounted for by the presence of some substances in the test plants as indicated in the quantitative analysis of the two plants. The observed decrease in the number of nematodes may be responsible for the increased in growth of the seedlings. Such decrease means fewer disturbances to the seedlings resulting in an unhindered growth (Vander-Borgett et al., 2019). The fewer number of galls observed in the plants treated with the various concentration of the extract may equally be an indication of an acquired resistance imposed by the extracts. However, in all the treatments observed, *Azadirachta indica* leaves extracts confers more protections on the cowpea seedling compare to *Vernonia amaegdalina* extracts, even though a significant improvement in plant growth was also recorded in all the parameters observed. The result therefore establishes the nematicidal potentials of the extracts obtained from the two plants. The efficacy of the plants extracts in controlling the nematodes population as well as improvement in plant growth can be attributed to the presence of some phyto- compounds as indicated in both the qualitative and quantitative analysis of the two plants.

The improvement in the root length and plant height of cowpea plant observed may be as a result of decrease in the number of nematodes which is known to cause a stunted growth on the infected plant as well as causing galling on the root which consequently affect the proper absorption of nutrient as well as water from the soil, this agreed with the work of Olowe T, (2021) on the use of root extracts of *Azadirachta indica* in the control of root-knot nematodes.

The study further shows that the potentials for treatment by the extracts of the plants are directly proportional to their concentrations. That at lower concentration of 100mg/ml the active ingredient with the nematicidal effect takes longer period to accumulate to the point of potency. This is in conformity with the work of Mahmood and Siddique (2018) on the effects of on the growth of cowpea and reproduction of *Rotelenchus reinformis.*

The experimental plants as shown in both the qualitative and quantitative analysis s (Table 3) is rich in various bioactive compounds such as flavonoids, tannins, Glycosides, saponin, and alkaloids. This agreed with the findings of Akhtar and Malik (2021), however phenols and glycosides were not detected in *Vernonia amaegdalina* and glycosides were not detected in Azadirachta indica, but the analysis revealed a high content of alkaloids in *Azadirachta indica* than in Vernonia Amaegdalina, perhaps that may be the reason for the observed differences in the efficacy of the two plants as indicated in the results (Table 1-7) This variation is not surprising because it is known that both genetic and environmental factors influence the content and composition of secondary metabolites in plants (Koupai-Abyazani et al; 1993).The geographical location of the sites of sample collection could also be responsible for variation in the chemical constituents of the plant. These compounds exhibit an array of biological activities including antifungal, anti-bacterial, and nematicidal effects. An in vivo study has confirmed the effectiveness of neem seeds extract in suppressing nematodes population in cowpea plants (Olowe et al., 2019). Ogunyemi et al., 2021, Adebayo et al., 2020, all reported an improved growth parameters such as root length, shoot height and fruit yield as well as enhanced soil microbial activity which contributes to nematodes suppression. Flavonoids in particular have been documented to disrupt nematodes development and reproduction. Research indicates that extracts from different parts of the plant including leaves and bark can have varying levels of toxicity against Meloidogyne specie. The significant differences observed in the various treatment groups in the experiment have indicated strong evidence that the extract of leaves of the experimental plants hold the potentials as nematicides.

## CONCLUSION

*Azadirachta indica* and *Vernonia amaegdalina* leaves extract have shown a promising potential as eco-friendly and sustainable alternative for controlling root-not nematodes infection in cowpea plant.

From the result obtained it can be concluded that the extracts of leaves holds the potentials for nematicidal activities. They contain such active substances which are harmful to nematodes and as such they could be used as nematicides Farmers may be encouraged to use the leaves either by mixing them with the soil through proper tilling method or by applying them directly y to the susceptible crops.

The result further establishes the effect of concentration on the treatment which indicated that at lower concentration the difference between the plant height and root lengths of all the cowpea plants treated with various extracts and those that are left untreated are insignificant. Likewise the same trend was observed with respect to nematodes population. The best solution to the nematodes problems is treatment with plant extracts which contain toxic substance to nematodes of which good examples are *Azadirachta indica and Vernonia amaegdalina*. The 400mg/ml concentration in both treatments have demonstrated the most potent nematicidal activity significantly reducing gall formation and nematode population comparable to infected untreated control.

The nematicidal activity of the extracts of the two plants represents a promising avenue for sustainable nematodes management in cowpea crops. While current research indicates its effectiveness, further studies are essentials to elucidate the specific mechanism involved and to determine optimal application rates and methods. By leveraging the natural properties of those plants, farmers may not only reduce nematodes infestation but also contribute to a more sustainable agricultural system. Continued exploration in to the plant-based nematicides could lead to more environmentally friendly practice in crop production as the quest

The Phytochemical screening of the experimental plant revealed the presence of a wide range of phyto-constituents some of which have been reported to possess nematicidal activity and which enables them to effectively suppress nematodes population while promoting plant health. However as indicated, further research is needed to optimize application methods and evaluates their long term impact under field conditions

## RECOMMENDATIONS

Based on the above findings the following are recommended

i) Further studies be carried out to identify the phyto-constituents that may be of possible effect using other plant models

ii) Farmers should be encourage to use this plants as an alternative method of controlling nematodes in order to reduce their menace on our crops

iii) Government and other research institutions should help in the productions of the pesticides with a view to commercializing it.

**REFERENCES**

Abubakar, U. (1999); Studies on the Nematodes of Cowpea *(Vigna unguiculata)* J... of the Savanna regions of Northern Nigeria and control of Meloidogyne Incognita using selected plant extracts and animal manures. Unpublished PhD thesis, Usmanu Danfodiyo University Sokoto P. 108

Adesiyan S.O. Cavenes, F.E.; Adeniji, M.O. and Fawole, B. (2010).*Nematode Pest* of *Tropical Crops.* Heinemann Educational Books (Nigeria) Ltd

Arshad, M. and Odeh, G.O (2014) The establishment of the Nigerian Sokoto Caliphate. An Inquest into the background History of the 1804 jihad in Hausa Land 210 years after. Al-qalam journal of Arts and Social Science, **2** (1): 61-79

Akhtar and Malik (2021) Role of Organic Soil Amendments and Soil Organisms in the ' Biological Control of Plant Parasitic Nematodes P. 26.

Baker K.F. and Look, R.J. (2018).*Biological Control of Plant pathogens.*W.H. Freeman and Company, San Francisco.

Broodier, B.B, Good J.M, and Jarwoski,C.A (2022) Population Dynamic of plant parasitic Nematodes in cultivated soil: Effects of summer cover crops in newly cleared land.Journal of Nematology **2**: 217-222

Egunjobi, O.A. (2015). Nematodes and Maize growth in Nigeria I. Population" dynamic of *Pratylenchusbranchyrus*in

and about the roots of maize and its effects on maize production at Ibadan.*Nematologica, **20(2):** 181-186*

Freedman, N. D.; Park Y.; Subar, A. F.; Hollenbeck, A. R.; Leitzmann, M. F.; Schatzkin, Abnet, C. C. (2017). "Fruit and

vegetable intake and head and neck cancer risk in a large United States prospective cohort study". *International Vournai of Cancer* **122** (10): 2330-2336.

Good, J.M ;Mintof, N.A. and Jaworski, C.A. (2018). Relative Susceptibility of selected cover Crops and coastal Bermuda grass to plant nematodes. *Phytopathology,* **55:** 1026-1030.

Hague, N.G.M. and Gowen, S.R. (2017).Chemical control of nematodes, p.131-178.*In* R.H. Brown & B.R. Kerry, eds. *Principles and practice of nematode control in* crops. Academic Press.

Isma'il, A., and Okey,I. (2012) Statistical and trend analysis of rainfall in Sokoto. International Research journal of engineering science, Technology and Innovation, **1** 161-174

Jibrilla, A.M., Jafar, M., and Choy L.K. (2019) Monitoring Vegetation Change in the dry land Ecosystem of Sokoto Northwestern Nigeria using Geo-informatics. Indonesian journal of Geography, **51 (**1): 9-17

Kasim,I., and Usman A.Y. (2021) Trend analysis of temperature and precipitation change in Sokoto State, Nigeria journal of Agricultural Economics and Rural Development 7(1): 941-951

National population Commission (2006) Population and Housing Census 2006 Nigeria

Ritter, M.Adamova SH (2023) Economic Importance of *Meloidogyne* in Europe and /Meditereriania. *Helmintological Abstracts, B.* No. 35. pp212

Shaukat S.S., A "Siddiqui, G.H. Khan and MJ. Zaki 2002, Nematicidal Allelopathic 'potential of Argemone mexicana a tropical weed PI soil **245** 239-247

Suadmadji, R.W (2019) Studies on the effects of tagetes spp on plant parasitic nematodes H.Veeman and N.V. Zonen,Wayemingen P312

Mahmood, I. and Siddiqui I.A. (2021). Effect of Phenol on the growth of Cowpea andReproduction of *Rotylenchulusreinformis. Mediterranean Journl of Nematolog*21:97-98.

Muyibi, S.A, Olorode,B.R, Onyelili,P.AOsunko, U.A; Muhammed B.Y and Ajagbonna, O.P (2000) Haematological and Histopathological changes of Senna occidentalis leaf extract in rats.Nigerian journal of Natural products and Medicine **4:** 48-52

Odihirifi R.A (2018) Screening of Some West African Cowpea for resistance to root -knot Nematodes meloidogyne, proceedings of the third planning conference on root-knot nematodes meloidogyne spp. International institute for tropical Agriculture Ibadan Nov. 16-20 2007.

Olowe, T. (2021) Control of root-knot nematode on Cowpea *(Vigna unguiculata) L* in Nigeria. In *Proceedings 3rd research Planning Conference on Root-knot* nematodes; Ibadan Nigeria.

Powell, NT. (2021) Interactions of plant parasitic nematodes, edited by Zuckerman, B.M.; Mai.

Sasser J.N.and D.W, Freckman (2017) A world perspective on nematology: The role of the society Pages 7-14 in: Vitas on nematology, J.A Veech and D.W.Dickson.eds society of nematologist.inc Hyattsville.MD

Shaukat S.S., A "Siddiqui, G.H. Khan and MJ. Zaki (2017), Nematicidal Allelopathic potential of Argemone mexicana a tropical weed PI soil **245** 239-247

Southey (2016).Laboratory methods for works with plant and soil Nematodes. Ministry of Agric & fish. Hm so London P. 202

Suatmadji, R.W. (2019). Studies on the effects of Tagetes species on plant parasitic nematodes*veeman and N.V. Zonen,* Wayemingen. 312.

Van Berkum, J.A. &Hoestra, H. (2019).Practical aspects of the chemical control of nematodes in soil, p. 53-154.*In* D. Mulder, ed. So/7 *disinfestation.*Amsterdam, the Netherlands, Elsevier.

Wang D.Y, S.Kumar and D.S Hedges, (2019) .Divergence time estimates for the early history of Animal phyla and the origin of plants, animals and fungi. Proceedings of the royal society of London **266:** 163-171.

Wallace, H.R. (2018). *The Biology of Plant parasitic Nematode.*, Edward Arnold Ltd.; London p. 280.

Whitehead, A.G. (2009). The distribution of root-knot nematodes ( *Meloidogyne* spp) in tropical Africa. *Nematologica*, **15:** 315-333.