



Effect of Crude Extracts of *Azadirachta indica* (Bark and Leaf) on Soil Microbial Load, Disease Manifestation and Growth Parameters of *Arachis hypogaea*

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Abstract

Groundnut is a vital legume crop susceptible to various diseases, affecting its yield and quality. Neem extracts have been explored for their biofungicidal properties. This study examined the impact of neem leaf, bark and bark+leaf extracts on soil microbial load and disease manifestation of groundnut. Groundnut soil was treated with neem leaf and bark extracts at varying volumes (5ml, 10ml & 15ml) weekly for four weeks. Number of colony forming units (5 CFU/ml, 10 CFU/ml & 15 CFU/ml) was conducted to determine viable microtoxic fungal cells in the groundnut soil. Growth parameters (plant height, leaf area, leaf length, leaf width, number of nodes and number of leaves) were determined. Visual assessment method was adopted for determination of percentage disease incidence. *Mucor* sp., *Rhizopus* sp., *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, *Fusarium* sp., *Candida* sp., *Microsporum* sp. and *Aspergillus fumigatus* were the fungi identified with varying number of colony forming unit based on the dilution factors. Chlorosis, leaf curling, leaf blight, damping-off and leaf spots were the visible symptoms at varying percentage disease incidence on the test groundnut. The combination of bark+leaf extracts exhibited dose-dependent disease suppression, but bark extract had higher efficacy. Neem leaf and bark extracts demonstrated antifungal potentials and enhanced growth parameters of groundnut plant. The bark extract had better performance and can serve as a sustainable alternative to synthetic fungicides and fertilizers.

Keywords: Groundnut, microtoxic, disease incidence, volumes, neem extracts and growth parameters

Introduction

Groundnut, also known as peanut, is a leguminous crop that plays a vital role in global agriculture due to its economic and nutritional importance. It is extensively cultivated in tropical and subtropical regions, particularly in countries such as India, China, Nigeria, and the United States (FAO, 2019) [11]. Groundnut is valued for its edible seeds, which are rich in oil and protein, making it a staple food source and a significant agricultural commodity (Janila *et al.*, 2013) [14].

Groundnut is a valuable crop cultivated for its edible seeds and oil. However, its production is often threatened by various pests and diseases, leading to significant yield losses. Effective management of these biotic stresses is crucial for maintaining groundnut productivity and ensuring food security. Examples among others are *Aphis craccivora* that feeds on the plant sap, weakening the plants and transmitting viral diseases such as peanut stripe virus (PSV) and groundnut rosette virus (GRV) (Kumar *et al.*, 2018) [18]. Aphid infestations can lead to stunted growth, yellowing of leaves, and reduced pod yield (Blackman and Eastop, 2000) [6]. White Grubs (*Holotrichia* sp.), which are soil-dwelling larvae that feed on groundnut roots and pods, leading to poor plant establishment and reduced pod quality (Saravanan *et al.*, 2015) [25].

Early Leaf Spot caused by *Cercospora arachidicola*: a fungal disease characterized by small, circular, dark brown spots on the leaves. The spots often have a yellow halo and can lead to defoliation and reduced pod yield if left unmanaged (Shew *et al.*, 1988; Kumar *et al.*, 2018) [18, 31]. Late Leaf Spot caused by *Cercosporidium personatum* which appears later in the growing season and produces larger, darker lesions than early leaf spot. This disease can also cause significant defoliation and yield loss, especially under humid conditions (Smith and Littrell, 1980; Blackman

and Eastop, 2000) [6]. Groundnut Rust (*Puccinia arachidis*): a fungal disease that produces orange-brown pustules on the undersides of leaves. Severe infections can lead to leaf drop and substantial yield reductions (Subrahmanyam *et al.*, 1985; Saravanan *et al.*, 2015) [25]. Sclerotium Rot (*Sclerotium rolfsii*); also known as stem rot or white mold, this disease causes wilting and death of the plant. It is characterized by the presence of white, cottony fungal growth at the base of the stem and sclerotia, which are hard, dark structures (Mehan *et al.*, 1994) [19].

Other fungi that have been reported to be pathogenic to groundnut include *Aspergillus niger* and *Fusarium* species, which cause significant damage through diseases like crown rot and leaf spot which do not only decrease crop yield but also affect the quality of the groundnuts, posing economic challenges to farmers ((Pandey, 1998; Nautiyal, 2002; Borgio, 2006; Pushpavalli *et al.*, 2014) [5, 20, 24].

Traditional disease management practices often involve the use of chemical fungicides, which has been proven to be problematic can lead to environmental pollution, development of resistant pathogen strains, and health risks to humans and animals (Biswas *et al.*, 2002; Pimentel, 2005; Carson *et al.*, 2007) [4, 7, 23]. Thus, researching on the alternative methods, such as the use of neem extracts, is important for sustainable agriculture (Isman, 2006) [13].

Neem (*Azadirachta indica*) has been recognized for its broad spectrum of medicinal properties and its significance in traditional medicine across various cultures (Govindachari *et al.*, 1998). Among its various parts, the bark and leaves have been particularly noted for their potential antimicrobial properties (Biswas *et al.*, 2002) [4].

The bark and leaves of the neem tree contain a plethora of bioactive compounds, including azadirachtin, nimbin, and quercetin, which exhibit strong antimicrobial activity against a range of pathogens (Pandey, 1998). Previous

studies have demonstrated the effectiveness of neem extracts in controlling plant diseases caused by bacteria, fungi, and viruses (Schmutterer, 2002) [26]. By leveraging these natural compounds, neem extracts could serve as a viable means to combat diseases affecting groundnut plants, potentially reducing the reliance on chemical pesticides and fostering sustainable agricultural practices (Koul, 2016) [16].

The potential of neem extracts as an antimicrobial agent against plant pathogens is promising, yet comprehensive studies on their application in groundnut disease management are limited (Schmutterer, 2002) [26]. Neem, a tree widely recognized for its medicinal properties, contains bioactive compounds such as azadirachtin, nimbin, and quercetin that exhibit strong antimicrobial activities (Talwar *et al.*, 1997 [33]; Koul *et al.*, 1990; Patil *et al.*, 2009) [22]. Other significant compounds include nimbin, nimbidin, salannin, and gedunin, which contribute to neem's wide range of biological activities (Koul *et al.*, 1990; Isman, 2006; Choudhary and Kumari, 2010) [9, 13]. Utilizing these natural compounds from neem could provide a sustainable and eco-friendly alternative to synthetic fungicides, thus reducing the environmental and health risks associated with chemical treatments (Aktar *et al.*, 2009; Khater, 2012) [1, 15].

Despite the known benefits of neem, there is a lack of comprehensive studies focusing on the use of neem extracts, particularly from the bark and leaves, in managing groundnut diseases thereby fostering adoption of more sustainable and environmentally friendly farming methods which would increase productivity and quality of groundnut crops, providing economic benefits to farmers and contributing to food security (Dhawan *et al.*, 1993; Singh, 1995; Chaturvedi *et al.*, 2014) [8, 28].

Researchers have shown that soil fertility is a fundamental element in agricultural productivity and sustainable food system. Overtime, conventional agricultural practices such as excessive use of synthetic fertilizers and continuous cropping leading to soil degradation, nutrient depletion and reduced crop yield (Pimentel, 2005) [23]. Organic amendments such as neem extracts provide alternative eco-friendly solutions by replenishing soil nutrients and improving soil structure for plant productivity which is in line with Isman (2006) [13].

Worlu *et al.* (2026) [35] reported that the impact of lime and lemon peel extracts on synergistic relationship between soil microbial load and growth parameters of soybean was effective as the result proved that the microbial load generally decreased as the concentration of lemon and lime peel extracts increased from 5mg l⁻¹ to 15mg l⁻¹ whereas, the microbial load in the control samples was generally higher than in the lemon peel extract treated samples. The decrease in microbial load as the concentration increased indicated an overall inhibitory effect of the extract on microbial growth which however, reflected on the growth parameters of soybean. This further showed that there was relationship between the amounts of fungal pathogens in the soil health status of a plant.

The application of neem extracts has also reported to positively influence the growth and yield of groundnut plants by improving soil microbial activity, nutrient cycling, and organic matter content, which are essential for long-term crop productivity (Anuradha and Sailaja, 2010; Bhadkaria and Vyas, 2011) [2, 3]. Neem's bioactive compounds not only provide disease protection but also enhance plant growth by improving soil health and nutrient

availability (Singh *et al.*, 2003) [29]. Reports have shown that neem-treated plants exhibit increased germination rates, higher biomass, and improved pod yields compared to untreated controls (Shukla and Kakani, 2016) [27]. This work was aimed at examining the effect of crude extracts of neem (bark and leaf) on disease manifestation and growth of groundnut.

Materials and Methods

Study Area and Materials Used

The research was carried out in the Plant Science and Biotechnology demonstration plot beside the Screen house at Faculty of Science, Rivers State University, Port Harcourt, Nigeria, which lies within latitudes 4°43'0743'07" and 4°54'3254'32"N and longitudes 6°56'0456'04" and 7°03'2003'20"E with a mean of annual rainfall of over 2000mm and mean temperature of 29°C (Tubonimi and Udonna, 2015) [34].

The materials used for the field work are as follows; Hoe and cutlass, Spade, Polythene bag, Groundnut seeds, Meter rule, Syringe, Neem bark and leaves and plastic bottles.

Materials used for the Laboratory work are as follows; sample collection polythene bags, hand gloves, test tubes, 90 ml disposable sterile petri dish, inoculating needle, spirit lamp, wire loop, auto clave, Aluminum foil, paper tape, ethanol, amoxicillin (antibiotics), beakers (200ml), Potato Dextrose Agar (PDA), salt, micro pipette, pipette tip, cotton wool, weigh balance, measuring cylinder, conical flask, distilled water, forceps.

Collection of Soil Sample

The soil samples were collected from ten different points (18m×18m) with the aid of farm spade which was used to dig a V-shaped hole to sample depth (3-6"). The soil cores were mixed (homogenized) in a clean plate and placed in a 17 × 17cm perforated polythene bags which weighed 4kg then, exposed to heat from sunlight between 27-35°C for five days. The collected samples were loamy soil. However, the soil samples were collected from the Botanical Garden, Rivers State University Port Harcourt and transferred to the Plant Science and Biotechnology demonstration plot beside the Screen house at Faculty of Science, Rivers State University, Port Harcourt where the research was carried out.

Collection of Groundnut Seed

Certified groundnut seeds (Variety: Samnut 26) were obtained from Agricultural Development Programme (ADP), Rumuodumaya, Port Harcourt, Nigeria on the 16th July 2024.

Planting Operation and Experimental Design

A completely randomized design was adopted. Groundnut seeds were planted in polythene bags of about 17cm in height and 17cm in width with 4kg of soil in each bag. Each bag had 3 groundnut seeds but One Week After germination (1WAG), 2 seedlings on groundnut were removed from each bag such that only one would be continually treated with the Neem crude extracts. This was to reduce nutrient competition between the plants. There were nine bags per treatment at different volumes (5ml, 10ml and 15ml) giving a total of 30 bags (sample size) including the control experiment.

Preparation and Application of Neem Crude Extracts

The crude from the Neem bark and leaves were extracted using conventional method of extraction. The Neem bark and leaves were placed in six plastic bottles (3 bottles for Neem bark and 3 bottles for Neem leaves) and 600ml of water was added to each bottle. The bottles were placed in a pot of clean boiling water for 30mins. This process was adopted to extract the crude from the Neem bark and leaves. The prepared crude extracts were measured (5ml, 10ml and 15ml) and inoculated into the polythene bags containing 4kg of soil at seven days interval for 3 weeks (Worlu *et al.*, 2026a)^[35]

Determination of Microbial Load of Soil Samples

The soil samples were collected from the bags (one from each treatment and concentration plus control, total of 10 soil samples) transferred to the Plant Science and Biotechnology demonstration plot, Rivers state university, Port Harcourt, Nigeria.

PDA (25.5g) was measured into a 1000ml of sterile conical flask. Thereafter 600ml of distilled water was added and shaken for 10 minutes. This was autoclaved for 15 minutes at 121°C, (1.02kgcm⁻³). The flask was later taken out of the autoclave and the temperature of the medium was allowed to cool down at 45°C before adding 250g of amoxicillin for the inhibition of bacteria growth. The PDA was then poured into disposable sterile petri dishes and swerved gently and was allowed to cool down to solidify before inoculation.

A saline solution of 900ml of water and 8.5g of NaCl was prepared. 9ml of the stock solution was pipetted into 40 test tubes and was corked with a cotton wool. The test tube alongside micro pipette tips were sterilized for 15 minutes at 121°C, (1.02kgcm⁻³) for 15 minutes. The sterilized test tubes were allowed to cool down at 40°C. Test tubes were arranged in three per rack and were labeled (10⁻¹, 10⁻² & 10⁻³). A pair of forceps was used to collect the soil sample and was placed into the first tube (10⁻¹), and was shaken vigorously to mix with the saline solution. 1ml was pipetted from the first test tube (10⁻¹) to another test tube on the same rack (10⁻²) and mixed with the pipette. 1ml was pipetted from it and poured into the third test tube (10⁻³) and was properly mixed with the pipette making it a threefold dilution. 1 ml of each concentration was inoculated into the plates (three replicates for each concentration). The plates were incubated at room temperature between 3 days to allow fungal growth. The fungal isolates were identified at the pathology laboratory, Department of Plant science and Biotechnology Rivers state university Port Harcourt, Nigeria.

Determination of Number of Colony Forming Units

Number of colony forming units (per unit volume) is a measure of viable microtoxic fungal cells in a sample. It is also used to calculate the number of CFU per volume (CFU/ml) of the original culture. The NCFU was derived as thus;

$$\text{NCFU} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plated}}$$

Where:

Number of colonies = number of colonies counted on the plate

Dilution factor = dilution factor used to prepare the plated culture (e.g., 10⁻³, 10⁻²)

Volume of culture plated = volume of culture actually plated (e.g., 0.1ml, 1ml)

Determination of Microbial Load

The microbial load was derived as thus;

$$\text{ML} = \frac{\text{NCFU} \times \text{dilution factor}}{\text{Volume of weight of sample} \times 10}$$

Where:

ML = microbial load

NCFU = number of colony forming units

Dilution factor = dilution factor used to prepare plate culture

(e.g., 10⁻³)

Volume of sample = volume (e.g., ml) of original sample

This formula takes into account the dilution factor and the volume of the original sample to calculate the microbial load. It also provides a more accurate calculation of microbial load considering the dilution factor and the sample volume.

Determination of Percentage Disease Incidence

Visual assessment method was used to estimate the quantitative damage that manifested on the plant leaves. The percentage disease incidence was derived as thus;

$$\text{DI} = \frac{X}{Y} \times 100$$

Y 1

Where;

DI = disease incidence

X = number of infected plant parts

Y = total number of plants assessed

This formula measures the number of new cases of a disease that occur within a population over a specific time or period, providing insights into the disease's spread/impact or providing a measure for disease incidence.

Data Analysis

Results were depicted as mean values and standard deviation of triplicate experiments. The data collected were analyzed for significant difference at 5% level of probability (p<0.05) using one sample T-test with Statistical Package for Social Science (SPSS) version 26.0.

Results

The fungal isolates identified were: *Mucor* sp., *Rhizopus* sp., *A. flavus*, *A. niger*, *P. notatum*, *Fusarium* sp., *Candida* sp., *Microsporium* sp. and *A. fumigatus*

Determination of Microbial Load in the Groundnut Soil

Table 1 shows number of colony forming units and microbial load on soil treated with Neem bark at (10⁻³g/ml) concentration. There were more occurrence of *Rhizopus* sp in 10ml and 15ml compared to control and nil in 5ml. *A. flavus* occurred more in 5ml compared to control and nil in 10ml and 15ml. More occurrence of *A. niger* in 5ml compared to 10ml and nil in 15ml and control. More occurrence of *P. notatum* in 5ml and 10ml compared to 15ml and control. More occurrence of *Fusarium* sp. in 5ml compared to 10ml and nil in 15ml and control, very high occurrence of *Candida* sp. in 5ml compared to 10ml and nil in 15ml and control. More occurrence of *Mucor* sp. in 15ml and control compared to 5ml and nil in 10ml, *Microsporium* sp. occurred only 10ml and nil in 5ml, 15ml and control and no occurrence of *A. fumigatus* in all concentration levels and control.

Table 1: Number of Colony Forming Units (NCFU) of Microflora on Soil Treated with Neem Bark at 10⁻³g/ml Concentration (CFU/ml)

Volume/Microflora	5 (CFU/ml)	10 (CFU/ml)	15 (CFU/ml)	Control
<i>Rhizopus sp.</i>	Nil	2.0×10 ⁻³	2.0×10 ⁻³	1.0×10 ⁻³
<i>A. flavus</i>	4.0×10 ⁻³	Nil	Nil	2.0×10 ⁻³
<i>A. niger</i>	4.7×10 ⁻³	1.0×10 ⁻³	Nil	Nil
<i>A. fumigatus</i>	Nil	Nil	Nil	Nil
<i>P. notatum</i>	2.0×10 ⁻³	2.0×10 ⁻³	1.0×10 ⁻³	1.0×10 ⁻³
<i>Fusarium sp.</i>	11×10 ⁻³	1.0×10 ⁻³	Nil	Nil
<i>Candida sp.</i>	36×10 ⁻³	6.0×10 ⁻³	Nil	Nil
<i>Microsporium sp.</i>	Nil	3.0×10 ⁻³	Nil	Nil
<i>Mucor sp.</i>	5.0×10 ⁻³	Nil	7.0×10 ⁻³	7.0×10 ⁻³

Table 2 shows number of colony forming units and microbial load on soil treated with Neem leaf at (10⁻³g/ml) concentration. There were same occurrence of *Rhizopus sp* in 5ml and control and nil in 10ml and 15ml, more occurrence of *Aspergillus flavus* in 15ml compared to control and 10ml and nil in 5ml, no occurrence of *A. niger* in all concentration levels and control, more occurrence of

P. notatum in 15ml compared to 5ml, 10ml and control, more occurrence of *Fusarium sp* in 5ml and 10ml compared to 15ml and nil in control, very high occurrence of *Candida sp* in 10ml compared to 5ml and nil in 15ml and control, more occurrence of *Mucor sp.* in 10ml compared to 5ml, 10ml and control and no occurrence of *A. fumigatus* and *Microsporium sp* in all concentration levels and control.

Table 2: Number of Colony Forming Units (NCFU) of Microflora on Soil Treated with Neem leaf at 10⁻³ g/ml Concentration (CFU/ml)

Volume/Microflora	5 (CFU/ml)	10 (CFU/ml)	15 (CFU/ml)	Control
<i>Rhizopus sp.</i>	1×10 ⁻³	Nil	Nil	1×10 ⁻³
<i>A. flavus</i>	Nil	5.0×10 ⁻³	6.0×10 ⁻³	2.0×10 ⁻³
<i>A. niger</i>	Nil	Nil	Nil	Nil
<i>A. fumigatus</i>	Nil	Nil	Nil	Nil
<i>P. notatum</i>	1.0×10 ⁻³	1.0×10 ⁻³	3.0×10 ⁻³	1.0×10 ⁻³
<i>Fusarium sp.</i>	7.0×10 ⁻³	7.0×10 ⁻³	3.0×10 ⁻³	Nil
<i>Candida sp.</i>	19×10 ⁻³	26×10 ⁻³	Nil	Nil
<i>Microsporium sp.</i>	Nil	Nil	Nil	Nil
<i>Mucor sp.</i>	4.0×10 ⁻³	11×10 ⁻³	5.0×10 ⁻³	7.0×10 ⁻³

Table 3 shows number of colony forming units and microbial load on soil treated with Neem leaves at (10⁻³g/ml) concentration. There were more occurrence of *Rhizopus sp* in 15ml compared to 5ml and control and nil in 10ml, same occurrence of *A. flavus* in 5ml and control and nil in 10ml and 15ml, *A. fumigatus* occurred only in 15ml and nil in 5ml, 10ml and control, no occurrence of *A. niger* in all concentration levels and control, more occurrence of

P. notatum in 10ml compared to 5ml and control and nil in 15ml, high occurrence of *Fusarium sp* in 10ml compared to 15ml and nil in 5ml and control, occurrence of *Candida sp* only in 15ml and nil in 5ml, 10ml and control, more occurrence of *Mucor sp.* in 15ml compared to 5ml, 10ml and control and no occurrence of *Microsporium sp* in all concentration levels and control.

Table 3: Number of Colony Forming Units (NCFU) of Microflora on Soil Treated with Combination at 10⁻³ g/ml Concentrations CFU/ml)

Volume/Microflora	5 (CFU/ml)	10 (CFU/ml)	15 (CFU/ml)	Control
<i>Rhizopus sp.</i>	1.0×10 ⁻³	Nil	2.0×10 ⁻³	1.0×10 ⁻³
<i>A. flavus</i>	2.0×10 ⁻³	Nil	Nil	2.0×10 ⁻³
<i>A. niger</i>	Nil	Nil	Nil	Nil
<i>A. Fumigatus</i>	Nil	Nil	2.0×10 ⁻³	Nil
<i>P. notatum</i>	4.0×10 ⁻³	8.0×10 ⁻³	Nil	1.0×10 ⁻³
<i>Fusarium sp.</i>	Nil	72×10 ⁻³	2.0×10 ⁻³	Nil
<i>Candida sp.</i>	Nil	Nil	7.0×10 ⁻³	Nil
<i>Microsporium sp.</i>	Nil	Nil	Nil	Nil
<i>Mucor sp.</i>	4.0×10 ⁻³	6.0×10 ⁻³	9.0×10 ⁻³	7.0×10 ⁻³

Examination of Disease Incidence and Percentage Disease Incidence for Neem Bark and Control at Four Weeks After Planting

Table 4 shows percentage disease incidence on groundnut plant with 15ml neem bark at 4WAP. Leaf blight had the highest disease incidence (34%), followed by damping off (2%) which recorded the least percentage disease incidence

and chlorosis, leaf curling and leaf spot had 0% disease incidence. Whereas, in the control leaf blight had the highest disease incidence (14%), followed by leaf spot (8%), chlorosis (6%), leaf curling (2%) which recorded the least percentage disease incidence and damping off had 0% disease incidence.

Table 4: Disease Incidence and Percentage Disease Incidence on Groundnut Treated With Neem Bark at 15ml 4WAP

Symptom	Assessed Plant Part		Not infected Plant part		Infected Plant part		% Disease incidence	
	Trt	Cont.	Trt	Cont.	Trt	Cont	Trt	Cont
Chlorosis	88	207	169	0	0	13	0	6
Leaf blight	88	207	186	58	30	30	34	14
Leaf curling	88	207	207	88	0	4	0	2
Leaf spot	88	207	208	88	0	17	0	8
Damping-off	88	207	210	86	2	1	2	0

Trt; Treatment, Cont; Control

Disease Incidence and Percentage Disease Incidence for Neem Leaves and Control at Four Weeks After planting

Table 5 shows percentage disease incidence on groundnut plant with 15ml neem leaf at 4WAP. Chlorosis has the highest disease incidence (17%), followed by leaf blight (16%), leaf curling (10%), leaf spot (10%) and damping off

(1%) which recorded the least percentage disease incidence. While in the control, leaf blight has the highest disease incidence (14%), followed by leaf spot (8%), chlorosis (6%), leaf curling (2%) which recorded the least percentage disease incidence and damping off had 0% disease incidence.

Table 5: Disease Incidence and Percentage Disease Incidence on Groundnut Treated With Neem Leaf at 15ml 4WAP

Symptom	Assessed Plant Part		Not infected Plant Part		Infected Plant Part		% Disease incidence	
	Trt	Cont.	Trt	Cont.	Trt	Cont	Trt	Cont
Chlorosis	80	207	66	194	14	13	17	6
Leaf blight	80	207	67	177	13	30	16	14
Leaf curling	80	207	72	208	8	4	10	2
Leaf spot	80	207	72	190	8	17	10	8
Damping-off	80	207	79	208	1	1	1	0

Trt; Treatment, Cont; Control

Disease Incidence and Percentage Disease Incidence for Combination of Neem Bark and Neem Leaves 4WAP

Table 6 shows percentage disease incidence on groundnut plant with 15ml combination of neem bark and leaf extracts at 4WAP Leaf spot has the highest disease incidence (7%), followed by leaf spot (6%) and leaf curling (6%) which

recorded the least percentage disease incidence while chlorosis and damping off had 0% disease incidence. Leaf blight has the highest disease incidence (14%), followed by leaf spot (8%), chlorosis (6%), leaf curling (2%) which recorded the least percentage disease incidence and damping off had 0% disease incidence.

Table 6: Disease Incidence and Percentage Disease Incidence on Groundnut Treated With Combination at 15ml 4WAP

Symptom	Assessed Plant Part		Not infected Plant Part		Infected Plant Part		% Disease incidence	
	Trt	Cont.	Trt	Cont.	Trt	Cont	Trt	Cont
Chlorosis	145	207	145	194	0	13	0	6
Leaf blight	145	207	136	177	9	30	6	14
Leaf curling	145	207	136	208	9	4	6	2
Leaf spot	145	207	134	190	11	17	7	8
Damping-off	145	207	145	208	0	1	0	0

Trt; Treatment, Cont; Control

Table 7 shows the combined effect of neem bark and leaf extracts on the physical growth parameters of groundnut at 4WAP. The result revealed that there were significant difference in all the growth parameters between the various concentrations (5ml, 10ml & 15ml) except the leaf width of groundnut at 10ml and 15ml. Generally, there was significant difference in the growth parameters between the treatments at all levels of concentration and control. In addition, there were significant differences in the physical

growth parameters of groundnut between the respective treatments; neem bark and leaf extracts and combined effects (neem bark + leaf extracts). The separate extracts (neem bark and leaf) had better impact on the growth parameters of groundnut than the combined (bark + leaf extracts). This may suggest that the combined treatment (neem bark + leaf extracts) had a suppressive impact of the growth parameters of groundnut 4WAP.

Table 7: Combined Effects of Neem Extracts (Bark + Leaf) on Physical Growth Parameters of Groundnut at 4WAP

Growth parameter/Vol. (ml)	Leaf Length (cm)	Leaf Width (cm)	Plant Height (cm)	Leaf Area(cm ²)	No. of Nodes (n)	No. of Leaves (n)
5	1.13±1.97 ^a	0.87±1.50 ^a	3.37±5.83 ^a	2.31±4.01 ^a	4.67±8.09 ^a	18.00±31.18 ^a
10	4.37±1.16 ^b	2.80±0.52 ^b	7.90±2.94 ^b	9.91±4.60 ^b	15.00±8.19 ^b	59.00±34.04 ^b
15	3.43±0.93 ^c	2.10±0.50 ^b	8.43±2.67 ^c	5.89±2.79 ^c	12.67±2.31 ^c	48.33±12.42 ^c
Control	3.53±3.08 ^c	2.07±1.81 ^{ab}	8.67±7.52 ^c	8.63±7.64 ^c	17.33±15.01 ^c	69.00±59.70 ^c

Means with different letters down the column are significantly different at (P<0.05)

Discussion

The examination of the effect of crude extracts of neem (bark and leaf) on soil microbial load and disease manifestation on groundnut revealed that propagules of soil-borne fungal pathogens such as *Mucor* sp., *Rhizopus* sp., *A. flavus*, *A. niger*, *P. notatum*, *Fusarium* sp., *Candida* sp., *Microsporium* sp., and *A. fumigatus* were present in the groundnut soil in the various carrying capacities. This finding is in agreement with Blackman and Eastop, 2000, Saravanan *et al.* (2015)^[6, 25] as well as Kumar *et al.* (2018)^[18] who reported that production is often threatened by various soil-dwelling larvae, pests and diseases, that feed on groundnut roots and pods leading to stunted growth, yellowing of leaves, poor plant establishment, reduced pod quality and significant yield losses. Borgio, (2006)^[5] alongside Pushpavalli *et al.* (2014)^[24] also reported the presence of *A. niger* and *Fusarium* species, which cause significant damage through diseases like crown rot and leaf spot which do not only decrease crop yield but also affect the quality of the groundnuts, posing economic challenges to farmers.

The number colony forming unit and microflora load of each of the fungal isolates responded differently to neem bark and leaf extracts at the various volumes and concentrations which might be due to their high reproductive potentials, fastidious growth and ability to multiply as well as colonizing the soil. This may be due to slow release and non-target or non-pathogen specific of the bioactive compounds to disrupt fungal growth and development through various mechanisms. This is in line with Schmutterer, (1990) who reported that neem extracts contain bioactive compounds such as azadirachtin, nimbin, and salannin which disrupt fungal growth and development through various mechanisms, including inhibition of spore germination and disruption of cell membrane integrity. However, Neem extracts are known for their broad-spectrum activity against a range of fungal pathogens, making them versatile tools in integrated pest management (IPM) strategies (Patil *et al.*, 2009)^[22].

The frequency of microflora load of the fungal isolate from the treatments were observed to be more compared to the control but was different and contrary in terms of disease manifestation on groundnut plant at the various volumes and concentrations. The relatively higher microflora load and fastidious growth noticed in the treated soil may suggest that the neem extracts served as bio-nutrients to soil-borne fungal pathogens in the bid to acclimatize thereby exhibiting some suppressive effect and resistance to the extracts. This corroborates with Bhadkaria and Vyas, (2011)^[3] who reported that neem extracts also contribute to sustainable agriculture by enhancing soil fertility and health via improvement of soil microbial activity, nutrient cycling, and organic matter content, which are essential for long-term crop productivity. Similarly, Anuradha and Sailaja, (2010)^[2] submitted that neem's biodegradability and low toxicity to non-target organisms make it an environmentally friendly option compared to synthetic agrochemicals.

The physical disease incidence or manifestation such as chlorosis, leaf blight, leaf curling, leaf spot and damping-off were attributed to the presence soil-borne fungal pathogens of groundnut which was more prevalent in the control than the treatments. The observed symptom is in line with Blackman and Eastop, (2000) and Kumar *et al.* (2018)^[6, 18] who reported early leaf spot (*Cercospora arachidicola*) and

Groundnut Rust (*Puccinia arachidis*) that fungal diseases are characterized by small, circular, dark brown spots on the leaves and the spots often have a yellow halo and can lead to defoliation and reduced pod yield if left unmanaged. In the manner, Mehan *et al.* (1994)^[19] and Saravanan *et al.* (2015)^[25] reported Sclerotium Rot (*Sclerotium rolfsii*); also known as stem rot or white mold that is characterized by the presence of white, cottony fungal growth at the base of the stem and sclerotia, exhibiting hard, dark structures that causes wilting and death of the plant. Borgio, (2006)^[5] as well as Pushpavalli *et al.* (2014)^[24] also reported a pathogenic attack on groundnut by *Aspergillus niger* and *Fusarium* species, which caused significant damage through diseases like crown rot and leaf spot thereby decreasing crop yield and quality of groundnuts, posing economic challenges to farmer.

Examination of the effect of neem extracts (bark and leaf) on groundnut plant growth parameters at 4WAP exhibited impacts and notable differences in number of nodes, plant height and other growth parameters when compared to the control. Neem bark significantly enhanced plant height, leaf length, leaf width, leaf area, number of nodes and number of leaves at the various volumes even as low as 5ml. Neem bark and leaf extracts hold significant promise for promoting healthy growth and yield. The positive impact on growth parameters of groundnut might be attributed to the increasing microbial activities experienced on the soil microflora load in the early stage of development of groundnut.

This observation corresponds to Anuradha and Sailaja, (2010)^[2] as well as Bhadkaria and Vyas, (2011)^[3] who reported that application of neem extracts had positive influence on the growth and yield of groundnut plants by improving soil microbial activity, nutrient cycling, and organic matter content, which are essential for long-term crop productivity. Singh *et al.* (2003)^[29] also submitted that neem's bioactive compounds not only provide disease protection but also enhance plant growth by improving soil health and nutrient availability. In like manner, Shukla and Kakani, (2016)^[27] reported that neem treated plants exhibit increased germination rates, higher biomass, and improved pod yields compared to untreated controls.

Worlu *et al.* (2026)^[35] reported that the impact of lime and lemon peel extracts on synergistic relationship between soil microbial load and growth parameters of soybean was effective as the result proved that the microbial load generally decreased as the concentration of lemon and lime peel extracts increased from 5mg l⁻¹ to 15mg l⁻¹ whereas, the microbial load in the control samples was generally higher than in the lemon peel extract treated samples. The decrease in microbial load as the concentration increased indicated an overall inhibitory effect of the extract on microbial growth which however, reflected on the growth parameters of soybean. This further showed that there was relationship between the amounts of fungal pathogens in the soil health status of a plant.

Conclusion

Effect of crude extract of neem (bark and leaf) on microflora load and disease manifestation of groundnut has been proven to exhibit antifungal activities and inhibited the spread of diseases on the foliar parts of groundnut. The combination of the extracts had higher efficacy in combating diseases as it had percentage disease inhibition as low as zero occurrence except for chlorosis (65%) even at

the lower volumes and concentrations. Neem extracts (bark and leaf) significantly enhanced plant height, leaf length, leaf width, leaf area, number of nodes and number of leaves at the various volumes even as low as 5ml. Therefore, research on the effects of crude extracts of neem (bark and leaf) on disease manifestation and growth of groundnut should be considered paramount to prevent economic and agricultural losses thereby maximizing quantitative and qualitative yield and income from groundnut plant.

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