NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 51 Number 3, December 2020 Pg. 134-147
Available online at: http://www.ajol.info/index.php/naj

RESPONSE OF VEGETABLE COWPEA (*Vigna unguiculata* L. Walp) TO SOME BIOPESTIDES FOR THE CONTROL OF BACTERIAL LEAF SPOT IN UMUDIKE, SOUTH EAST NIGERIA

Opara, E. U. and Abengowe, C. C.

Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike
Corresponding Authors' email: euopara22@gmail.com

Abstract
An experiment trial was conducted in Michael Okpara University of Agriculture, Umudike to assess the efficacy of some leaf extracts (*Vernonia amygdalina, Gongronema latifolium* and *Ocimum gratissimum*) in the control bacterial leaf spot disease of vegetable cowpea). Also, their effects on disease severity and disease incidence, growth and yield performance were also investigated during the 2016/2017 and 2017/2018 cropping seasons. Four varieties of vegetable cowpea were used (Black vegetable cowpea, Brown vegetable cowpea, Ife brown and Ife-143), were tested to susceptibility by bacterial leaf spot pathogen with a view to identify the most resistant variety that can be adopted by farmers. The experiment was laid out in a randomized randomized complete block design (RCBD) with three replicates and plant the plant extracts were applied two weeks after planting. Isolation and identification were also done by collecting diseased leaf plants from the field to the laboratory by performing pathogenicity tests both invivo and invitro to confirm the identity of the casual organism. Similarly, some biochemical tests were conducted (Gram-negative, catalase, oxidase, indole, maltose, xylose and glucose) to further confirm the identity of the pathogen. Based on these tests conducted, the pathogen was identified as *Xanthomonas axonopodis* pv. *vignicola*. Data obtained from the two years field trials on growth parameters (plant height, number of branches, number of leaves per plant, stem girth) and on yield performance including thoe of disease (incidence and severity) were subjected to statistical analysis (Fishers Lsd P≤0.05). Results obtained from the field showed that significant differences (P≤0.05) existed among the different varieties on one hand and among the leaf extracts on the other hand (74-80% success in reduction) for instance lowest percentage disease incidence on vegetable cowpea was recorded with *Vernonia amygdalina* treated plants (10%) when compared with the control (80%). While reduced disease severity score was recorded for the *Gongronema latifolium* (2.00) compared with the control (8.90) on Ife-143 plants. These significantly enhanced yield and yield components (P≤0.05).

Keywords: Plant extracts, pathogenicity, isolation, identification, severity, and incidence

Introduction

*Vigna unguiculata*L. Walp also called vegetable cowpea is a dicotyledonous plant, which fits into the family of Fabaceae. Vegetable cowpea is member of *Vigna*, Genus *unguiculata* which is a latin word. According to Ano and Ubochi (2008); it originated from West and Central Africa but widely grown in Latin America and South-East Asia. It is an important source of livelihood to Nigerians. The immature seeds, pods and leaves of legumes can be eaten fresh. *V. unguiculata* is a staple food crop of significant economic importance in Nigerian and worldwide. Its cultivation provides social and economic benefits; cash to smallholder farmers due to its many uses. It is also cultivated solely in Nigeria though it's not among the crops frequently researched on (Ano and Ubochi, 2008; Akpan, 2014). However this all important crop is seriously threatened by so much biotic challenges and constraints.

Bacterial leaf spot occurs in all vegetable cowpea growing parts of Nigeria (Emechebe and Lagoke, 2002). Symptoms may start as spots on leaves which enlarge and become necrotic. Spots may be enclosed by an area of yellow discoloration; lesions merge to give plant a burned appearance, otherwise called blight, with time dead leaves still remain attached to the plant, circular, sunken, red coloured lesions are present in the pods; pods lesions may discharge bacterial fluids during wet conditions. Disease incidence of bacterial blight is linked to seed-borne nature of the pathogen with secondary spread occurring by rain. The bacterium lives in the soil for up to 8 months and in debris for more than 8months. (Okechukwu and Ekpo, 2008), which causes huge yield loss of more than 64% in some part of West

African (Sikirou, 1999) when highly susceptible cultivars are grown, the crops are totally destroyed (Emechebe and Shoyinka 1985).

Integrated pest management program (IPM) also encourages the use of plant materials for disease control which is among the possible strategy. Natural plants products are important sources of new agricultural chemicals used to check insect pests and plant diseases (Amadioha, 2003). The natural substance has useful properties, they are quite specific and cause little disturbance to the natural balance between living organisms. They are cheap and can be produced by farmers and local sources. They are often harmless to human and animal and rarely toxic to plant when compared to artificial fungicides (Opara and Wokocha, 2008). Plant species like *Gongronema latifolium, Vernonia amygdalina* and *Ocimum gratissimum* have been reported to be encouraging species as crop protection (Stoll, 2000; Opara and Wokocha, 2008). These crop protection features have been ascribed to the following such as; peptides, alkalonoids, essential oils, phenols and flavonols which serves as important mechanisms in these plants (Okigbo and Igwe, 2007). Medicinal plants such as *Vernonia amygdalina, Gongronema latifolium* and *Ocimum gratissimum* has proven to offer numerous medicinal assets. These medicinal assets exercise bacteriostatic and bacteriocidal effects on several bacteria. Karuna and Khan (1993) reported that plant extract obtained from *Ocimum eucalyptus* inhibited the growth of pathogen of bacterial blight. *Ocimum sativum* effectively controlled bacterial blight of cowpea caused *Xanthosomas axononopodis* pv. *vignicola* when undiluted extract is sprayed on the crop and effectiveness was decreased under dilution (Amadioha and Obi, 2009). Similarly, Prasad and Alankararao (1987) evaluated the antimicrobial effects of several important oils from a variety of speciesof *Ocimum.* All the samples showed antibacterial activity against gram positive and gram negative bacteria. Opara and Wokocha (2008), observed that aqeous extracts *Azadirachta indica* seed, *Piper guineensis*, *Citrus sinesis* and *Chromolaena odorata* were effective in inhibiting the growth of bacterial leaf spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) *in vitro* and *in vivo.* The inhibitory effect attached to certain plant extracts of seeds such as; paw paw, water melon, orange peels and moringa leaves used for the management of leaf spots of bacterial origin (Opara and Wokoha, 22008). However, several authors have tested the effect of diverse plant extracts usedas bio-pesticides for reducing different pest species. Cashew (*Anacardium occidentale*) plant extracts has been stated to be effective against post flowering insect pests and pathogens of vegetable cowpea (Amatobi, 2000; Okparake *et al.,* 2001). Organic soil amendments, composed organic material or extracts applied to seeds and liquid extracts from composed material sprayed on leaves, have been found to restrain the development of fungal leaf pathogens (Stoll, 2000).

Materials and Methods

Experimental site

The trial was carried out at the Experimental Farms of College of Crop and Soil Sciences of Michael Okpara University of Agriculture, Umudike (MOUAU) in the 2017 and 2018 cropping seasons. Umudike is a farming community located between longitude 07°33E, latitude and 05°29N, altitude 122m with annual rainfall of 2177mm, 72% relative humidity, monthly and ambient temperature of 17°C to 36°C in 2018 from May - September.

Experimental design

The field lay out was designed in a Randomized Complete Block design (RCBD) and was replicated three times;b four varieties of vegetable cowpea; namely: Black vegetable cowpea (Akidielu), Brown vegetable cowpea (Akidiala), Ife brown (brown cowpea) and Ife 143 (small brown cowpea); three plant extracts were used; *Vernonia amygdalina* (bitterleaf), *Gongronema latifolium*(Bush buck)*, Ocimum gratissimum* (scent leaf) and a control (sterile water). Soil was collected from the Research Farm site was augumented with organic manure after being cured fo two weeks. (Dawson *et al.,* 1965).

Soil analysis

Soil Samples were collected from pits 0-30cm depth, bulked into composite sample and taken to the College Soil Science laboratory for analysis to determine the physico-chemical properties of the experimental site. At the laboratory, composite samples were air-dried at a room temperature of 27°Cfor three days, crushed and sieved using 2mm aperture. (Gee and Bauder, 1986) The parameters considered included the particle size distribution by hydrometer method (Gee and Bauder, 1986). Soil pH8.5 was determined using Pye Unican model MK2 pH meter in a 1:2:5 soil/water suspension ratio. Organic carbon was determined by Walkeley-Black wet method(Nelson and Sommers, 1982). Total nitrogen was determined by micro-Kjeldahl distillation technique and available phosphorus was determined by flame photometer, while cation exchange capacity (CEC) was determined by Amonium acetate saturation method (Roades, 1982).

Source of plant materials

Seedplanting materials weresourced from the National Horticultural Crops Research Institute (NIHORT) Mbato, Okigwe Sub Station, Imo state. The plant materials consist of four varieties of *V. uniguiculata*. Also plant materials used for plant extracts weresourced from local communities of Umudke and Umuariaga which included: *V.amygdalina* (bitter leaf), *G. latifolium* (bush buck) and *O. gratissimum* (scent leaf).

Preparation of plant extracts

Fresh leaves of *V. amygdalina*, *G. latifolium* and *O. gratissimum*were thoroughly washed in running tap water and rinsed with distilled water (Effraim *et al*., 2000). They were air dried for 48hours to a constant weight and milled to a fine paste with the aid of a

Binatone blender (Model BLG-401). The solvent used for preparation of the extracts was sterile water. 250g each of the milled paste of *V. amygdalina*, *G. latifolium*, *O. gratissimum* was added in 200ml of sterile water for 10 minutes in a 500ml beaker to obtain a suspension by stirring vigorously with a glass rod.(Amadioha, 2003). Later sieved through two layers of cheese-cloth and finally filtered using Whatman no.1 filter paper.

Application of plant extracts

Suspension of each plant extracts was applied using hand-held sprayer (Opara and Wokocha, 2008) at 20ml/plant stand at two weeks intervals after inoculation on plant leaves and application was done till eight weeks after planting (8WAP). A control experiment was also conducted using sterile water. The application was done after sunset (evening period) to reduce the rate of solar decomposition of the volatile active ingredient.

Assessment of percentage disease incidence and disease severity

The plants were examined for disease symptoms from four weeks after planting (4WAP) and the number of plants and parts infected were recorded until eight weeks after planting (8WAP). Assessment was done using percentage incidence formula:

$$\text{Percentage Disease Incidence (\%)} = \frac{\text{Number of plants infected in the sample}}{\text{Total Number of plants examined in the sample}} \times \frac{100}{1}$$

Disease Severity

Severity score was based on the scale of 1-6, a modified scale by Opara and Wokocha (2008).:

1 - Leaves without bacterial spot

2 - A few bacterial spots on the leaves, about 5% of the leaves covered

3 - Bacterial spots join together to form necrotic lesion, covering about 25%

4 - Bacterial spot enlarged and extended to the leaf margin or about 50% surface covered

5 - Bacterial spot tear and leaf partially rotten, covering about 75%

6 - Leaf collapsed/completely rotten, turn apart and may fall off covering 100%

Assessment of growth parameters

Data for assessment for growth and yield were collected on the following parameters;

i. Vine length or Plant height (cm): this was done using a measuring tape.
ii. Number of leaves.
iii. Number of branches.
iv. Stem diameter (cm): this was done using a measuring tape.
v. Number of pods: this was done by counting the number of pods.
vi. weightof1000 seeds (g)
vii. Yield weight at harvest (Kg).

Laboratory Experiments

The laboratory work was carried out at the Crop and Soil Sciences laboratory, Michael Okpara University of Agriculture, Umudike and Central laboratory of the National Root Crop Research Institute (NRCRI), Umudike.

***Sterilization of glass wares and inoculation tools*:** All glass wares and metal tools used for the experiment were sterilized by autoclaving at 121°C/15psi for 30minutes before use while the chamber was mopped with 70% absolute alcohol to avoid contamination.

***Preparation of agar culture medium*:** The culture medium was prepared according to manufacturers' recommended instructions. Twenty-eight grams (28g)of Nutrient agar (NA)obtained from Titan Biotech Ltd. (Bhiwadi-301019, Rajasthan, India) was dissolved in 1litre of distilled water in a conical flask (Fahy and Hayward, 1983), it was shaken thoroughly to obtain a homogenous mixture. The mixture was autoclaved at121°C/15psifor 30minute, allowed to cool down to 45°C after which 15ml was dispensed into sterilized 9cm diameter Petri-dishes. The agar plates were allowed to solidified and were turn upside down to enhance drying at 28-30°C for 8hours before use. The bacterial suspension was streaked onto the Nutrient agar (NA) in Petri dishes using a flamed wire loop after which the culture was placed in an incubator at 30°C for 24hours. The culture colonies obtained after 48hours was sub-cultured severally to obtain pure bacterial colonies. A standard bacterial inoculum was obtained by serial dilution plating by addition of 1ml of sterile water into the culture colonies and then using wire loop to mix the water into suspension which was adjusted to the concentration of 10^8cfu/ml (colony forming unit per meal).

***Isolation of pathogen*:** Diseased leaves of *V. unguiculata* were collected from infected plants growing in the field. It was washed under a running tap, 1-2mm taken from the advancing edge of the legion with a sterilized scapel. The cut section was rinsed in three changes of sterile water and macerated with a glass rod in a Petri dish with a drop of sterile water to form suspension according to Bradbury (1970). The suspension was allowed to stand for 30minutesin order to allow bacterial cell to multiply. The suspension was streaked into the solidified agar plate with the aid of a flamed rod and cooled wire loop and plates were incubated at 30°C in the laboratory for 24hours, after which single colonies from the 24hours old culture were picked with a sterile wire loop and streaked in a zig-zag fashion unto fresh culture in Petri dishes. The procedure was repeated by sub-culturing thrice to obtain pure cultures.

***Preparation of Pathogen inoculum*:** Bacterial inoculum was prepared from 24 hours old cultures by washing bacterial colonies on agar plates with sterile water into McCartney bottles and adjusting the concentration density of the inoculum to 10^8 cfu/ml

(colony forming unit per meal).

***Identification of pathogens*:** Pathogenic organisms isolated from diseased leaves of *V. Unguiculata* were identified based on pathogenicity test, morphological and biochemical tests using Bergy's Manual of Determinative Bacteriology (Buchaman and Gibbons, 2004) and those with corresponding characteristics of identification.

Pathogenicity test
Ten Pots containing two weeks old seedlings of *V. Unigiculata* were raised in 10 liters perforated buckets filed with sterilized soil up to three quarter of the bucket. Seedlings were first pre-inoculated two weeks before using bacterial inoculums at a concentration of 10^8cfu/ml followed by the application of plant extracts. The seedlings were inoculated by spraying the bacterial inoculum on the leaves using hand atomiser in the evening (6-6:30pm). The younger leaves and emerging shoots were also sprayed until there was a run-off; the inoculated seedlings were later covered with a transparent polyethene bag to create a high humid condition 70-80% and allowed for 48hours at 25-27°C for the bacterial pathogens to incubate and the seedlings were observed daily for 4-6 days for symptoms of bacterial spots and re-isolation made from disease leaves. (Jones *et al.,* 2000).

***Morphological and biochemical tests*:**
The direct culture method developed Cheesbrough, (2000) was adopted in this regard, the infected leaves were surface sterilized in 70% ethanol few seconds. Following this and working under the aseptic condition of the inoculation chamber, the leaves were cut out in bits containing boundary portions of the leaf between the infected and healthy parts of the leaf. The cut-out bits constituted the innocula which were carefully placed on the surface of sterile solid Nutrient agar. Three leaf bits were inoculated onto each 11cm plate and the plates were covered, labelled and incubated at ambient temperature (28±2°C) for 48hours. They were examined for colony growth. From this culture, distinct colonies were collected using flamed wire loop, and transferred to sterile solid Nutrient agar (NA) plates by streaking technique. They were incubated as described earlier and observed for colony growth and the emergence of uniform colonies in the sub cultured plates were recorded, to prove purity on pure cultures; each isolate was used for characterization and subsequent identification.

Characterization of bacteria isolates
Each bacterial sample in a pure culture was characterized by study of their respective general and peculiar characteristics which enabled their phenogenic identification in line with existing taxa based on Bergy's Manual of Determinative Bacteriology (Buchaman and Gibbons, 2004). A four step identification techniques were used:

Colony morphological features
The features of the colonies in the pure culture were examined closely and visually and the observed colony characteristics recorded against each isolate including the extract of growth, elevation; pigmentation, colours, form of margins, consistency etc.

Microscopic features
The isolates were subjected to microscopic examination to determine among other features, their respective Gram reaction (positive or negative reactions), to specific dyes (stains) to show the presence or otherwise of features like spores, flagella etc. and in addition the above, the shapes and arrangement of the bacteria cells were observed. Every observation was recorded against the isolates individually.

Biochemical tests
Tests were conducted on each bacterial sample based on their negative or positive to some biochemical reactions including their ability to produce enzymes such as catalase, oxidase and urease and also their abilities to reduce sugars, in addition to Indole production from methyl red and Voges Proskeur were also included in the tests. All observations were recorded accordingly (Cheesbrough, 2000; Cheesbrough, 2002; Buchaman and Gibbons, 2004).

***Indole test*:** was conducted by inoculating Tryptophan broth with the test organisms and incubated at 37°C for 24hours by adding 0.5ml of Kovac's reagent and gently agitating, examining the upper layer of the liquid, and tested if red colour would appear in few seconds according to Snell (1999) and Mac Faddin (2000).

***Oxidase test*:** was conducted using test tube method; grown on a fresh culture of 24hours of bacterial culture in 4.5ml of nutrient broth (NA) then addition of 0.2ml of 1% α-naphthol and then by adding 0.3ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents) to the overnight broth culture agitated vigorously to ensure mixing and thoroughly oxygenation of the culture, then the colour changes were observed within 30 seconds

***Catalase test*:** using slide or drop catalase method by placing a microscope slide inside a Petridish, using a sterile inoculating loop, small amount of the test organism from a well-isolated 24hour old culture colony was placed into the microscope slide, (caution was applied not to pick agar). Using a dropper one drop of 3% Hydrogen peroxide (H_2O_2) was placed into the organism on the microscopic slide, the culture was not mixed immediately but was covered with a lid to limit aerosols and the formation of bubbles against a dark background was observed which enhanced readability. Positive reaction was evident by immediate effervescence (bubble) formation. (Mac Faddin, 2000; Duke *et al.,* 1972).

***Urease test*:** By inoculating slope heavily from an 24hour culture over the entire surface by streaking the

surface of the agar in zig-zag manner and later incubated the inoculated slope with loosened caps at 35±2°C for 48hours, examined for colour change after 6hours and after overnight incubation (longer period was found necessary and was expected). Appearance of bright pink colour on the slant was expected. The observations were recorded. (Collins *et al.,* 2004; Mac Faddin, 2000; Brink, 2013).

***Carbohydrate utilization tests*:** This test involved the ability of the bacterial isolates to utilize various sugars as energy source and is marked with the ability to produce acid (as shown by colour change of indicator in the liquid medium) as well as the ability to produce gas (as shown by the presence of entrapped air bubbles in an inverted Duliam tube in the medium). The test sugars included glucose, sucrose, maltose, lactose, mannitol and xylose. The observations were also recorded.

***Identification of bacteria isolates*:** Identification of the bacteria isolates from the infected *V. unguiculata* leaves were based on matching characteristics with existing taxa in standard bacterial mannuals. In this regard, the obtained characteristics were matched against those available taxa in the Bergy's Mannual of Determinative Bacteriology and the isolates with matching characteristics were recorded and identified.

Statistical Analysis

All the data collected were statistical analysed using SAS model (2008) and analysis of variance (ANOVA) determined along with significant means which were separated using Fishers Least Significant Difference (LSD) at 5% level of probability (P≤0.05).

Results and Discussion

Effect of Plant Extracts on Growth Parameters of Vegetable Cowpea in the 2016/2017 Cropping Season

Plant Height*:* Results obtained (Table1) showed that variety (var.) Black vegetable cowpea when treated with *G. latifolium* gave the highest plant height (35.83cm) which was statistical different (P≤0.05) from the ones treated with sterile water or control (5.67cm). This was followed by Ife brown (35.00cm). Brown vegetable cowpea had the lowest (14.00cm).

Number of branches*:* in this regard, it was found that var, Ife143 had the highest number (15.08) when the plants were treated with *O. gratissimum* compared to those treated with control (6.92) as shown in Table1 while var. Black vegetable cowpea scored the lowest (14.58) with same extract treatment which was not different statistically from other Ife brown cowpea variety (P≤0.05).

Number of leaves: In this case Black vegetable cowpea variety had the highest number (17.17) followed by Ife brown cowpea (14.50) when plants received treatments with *O. gratissimum* which was not statistically different but statistical difference existed among other varieties at P≤0.05 . However, when same varieties were treated with control plants perform poorly with 4.42 and 2.83 for Black and Ife brown cowpeas respectively.

Stem diameter (cm): Plants treated with various plant extracts produced no significant effects at probability of 5% (Table1)

Disease Parameters

Disease severity: During the 2017 wet season, the result obtained with disease severity showed that there were significant differences among the cowpea varieties after treatments with the different extracts (Table 2). Ife143 variety recorded the least interms of severity (2.00) with *G. latifolium* application when compared with the control (8.17). Next to *G. latfolium* was *O. gratissimum* (3.67).

% disease incidence: Table 2 showed that Brown vegetable cowpea variety was the most resistant (4.5) when plants were treated with *G. latifolium* which was significantly different from var. Brown vegetable cowpea (76%) with treatment (P≤0.05).

Yield and Yield Attributes

Number of Pods: Data (Table2) showed there were some significant differences between varieties in terms of number of pods for instance, var. Black vegetable cowpea had highest number of pods (9.06) with *G. latifolium* followed by brown vegetable cowpea (8.16) this two varieties were found to be superior to the other varieties.

The 1000 seed weight per plot: The results showed it was the same Black vegetable cowpea (12.76) that recorded the highest weight with regards to 1000 seeds when *G. latifolium* was applied while Brown vegetable cowpea lowest (4.40) with *O. gratissimum* as the extracts treatment.

Seed Weight per Hectare (Tons/ha): Ife brown recorded the highest seed weight per ha. (30.33kg) with *G. latifolium* extract application and was significantly different statistically followed by Black vegetable cowpea (28.14kg) when plants were treated with *G. latifolium* and Ife 143 (4.92kg) when control was applied.

Effect of Plant Extracts on Growth Parameters during 2018 Cropping Season

Results of effect of extracts on the cowpea varieties and growth parameters in the 2018 cropping are summarized in Table 3. When the plant extracts were considered for instance in the case of *V. amygdalina* treated plants, Black variety recorded the best in plant height (32.08cm) which was followed by Ife brown (26.42cm) while the control (sterile water) treated plants produced significantly (P≤0.05) lesser plant height with 4.00cm and 5.42cm for black cowpea and Ife brown respectively. Number of branches produced no significant effect (P≤0.05) among all the extracts treated plants when compared with the control treatments on all the four cowpea varieties. However, considering number of leaves significant differences existed among all the varieties but *O. gratissimum* treated plants

produced crops with higher number of leaves with black cowpea variety having the best (46.08) followed by Brown cowpea (38.75) untreated plants (control) produced significantly poor plants with lesser number of leaves in all the varieties Table3). Stem diameter parameter was consistently not significant among the varieties and among the different plant extracts considered (P≤0.05).

Effect of Plant Extracts on Disease and Yield Attributes of Vegetable Cowpea during 2017/2018 Cropping Season

Table 4 showed data on disease severity was least with *V. amygdalina* extract treated plants and the least disease in this parameter Ife143 was with the least (3.42) followed by the black cowpea (4.00) while control experiment did not produce any significant effects in all the varieties treated when compared with *V. amygdalina* and *G.latifolium* treated plants. When the data on percentage disease incidence was considered *O. gratissimum* treated plants performed best with Brown cowpea scoring the least (3.75%) Percentage disease incidence followed by black cowpea (4.33%). *G. latifolium* treated crops were also significantly effective for the variety Brown cowpea (5.00%) while untreated control plants produced significantly higher disease incidence ranging from 25.75% to 30.25% incidence for brown cowpea and Ife brown/Ife 143 respectively when compare with plants treated plant extracts (Table 4). Effect of the Plant extracts on the number of pods of the cowpea varieties showed that *O. gratissimum* treated plants produced significantly the highest when compared the untreated control (Table 4). In the case of 1000 seed weight per plot, the data produced similar results as in the case of pod weight with *O. gratissimum* treated crops producing the bet seed weight in comparism with the untreated control plants (P≤0.05). Regarding seed weight (tons per hectare) black cowpea produced the highest (47.92 t/ha) with *G. latifolium* treated crops, similarly *V. amygdalina* produced good results P≤0.05 when applied on black cowpea (46.08t/ha).

Morphological Characterization of Bacterial Spot Pathogen in the Laboratory

Table 5 summarized the result of the Identification and preliminary tests for the bacterium isolated from diseased plant leaves. The characteristic features of the isolate were compared with the description of Bergey's Manual of Determinative Bacteriology (Buchaman and Gibbsons, 2004; Bradbury, 1986). The laboratory experiments revealed that the bacterium isolated from the infected leaves of *V. unguiculata* was found to be gram negative rod bacterium based on the characteristic features, the isolated bacterium was suspected to be *Xanthomonas axonopodis* pv *vignicola* (Table 5).

Pathogenicity Test

A pathogenicity tests was carried out where the bacterial isolate was inoculated onto healthy young shoots of vegetable cowpea. The results of the pathogenicity test conducted on young shoots of *V. unguiculata* showed that the organism induced spots on the inoculated young *V. unguiculata* seedlings after 7days in conjuction with Koch's postulate.

Soil Analysis and Characterization of Experimental Site

The physical and chemical properties of the soil site are presented on Table 6. Soil test conducted showed that the experimental soil was sandy loamy, moderately acidic with pH 5.4, sand, silt and clay particles of 72.79%, 10.40% and 16.81% respectively. It has low nitrogen level of 0.042mg/kg and exchangeable calcium of 2.38cmol/kg.

Natural plants products are important sources of new agricultural chemicals used in the control of insect pests and plant diseases (Amadioha, 2003). The natural substance have useful properties, they are quite specific and cause little disturbance to the natural balance between living organisms. Inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials (Amadioha and Obi, 1999; Okigbo and Ajale, 2005; Okigbo *et al*., 2005). This study however supports the use of *Vernonia amygdalina, Gongronema latifolium, Ocimum gratissimum* in as a biopepticides reducing plant diseases and as well as a therapeutic agent and can explain the long history of these plants as botanical agents.

Agrios, (2005) reported that in the early stages of infection by bacterial leaf spot pathogen, incidence may increase rapidly with time, and. also that disease severity of individual plants may be low initially but subsequently increase with time. The steady increase in disease incidence and severity of bacterial spot during the growth period could be attributed to increase in inoculum load and virulence of the casual pathogen on its host as duration of infection increases. This is in line with the observation made by Wasihum and Flagote, (2016).

The varietal differences in yield and yield component and disease resistance may be due to genetic variation among varieties. Other workers have reported the difference in the performance of cowpea varieties (Yayock *et al.,* 1977; Ayaz *et al.,* 2004; Kamara *et al.,* 2010). In this present study black cow pea and Ife brown had better resistance to bacterial leaf spot than other varieties besides it had more pods, seeds with higher weights records when compare with other varieties.

According to Wagner, (2004) the pathogen *Xanthomonas axonopodis* pv. *vignicola* itself is seed borne, which can then spread to other nearby plants after the seedling begins to grow through splashing water and overhead irrigation. Seed is the primary inoculum source of the pathogen which results to either pre or post–emergence seedling infection and subsequent mortality (Ganiyu *et al.,* 2017). Spread of the disease is moderately fast if water splashing is highly prevalent. However, this pathogen *Xanthomonas axonopodis* pv. *vignicola* is highly dependent on wet conditions, so if

these conditions are not met, the pathogen's distribution will be highly deterred (Wagner, 2004). Most diseases thrive best under high relative humidity, which correlates with high rainfall pattern and atmospheric temperature that are found in humid forest of Southern Nigeria.

The study revealed also that the disease incidence and severity progresses at different growth stages of the crop. The lowest percentage incidence and severity was observed at the early stage of shoot growths while the highest incidence and severity were observed at fruit formation stage. This also agrees with the report of Emechebe and Lagoke (2002) who observed that disease incidence and severity are usually higher at fruit stage of most cowpeas leading to yield loss and similar observation was also made in this study.

Disease symptoms were mainly as large brownish necrotic lesions on the margin and centre of the leaves. The bacteria infected the stem, causing crack, canker and leaves that die may remain attached to the plant, circular, sunken, red brown lesion may be present in the pods; pods lesions may ooze bacteria fluids during humid conditions, causing water-soaked spots. The bacterium may also survive in the soil for up to 8 months and in debris for longer periods, and is favored by rainfall. (Okechukwu and Ekpo, 2008). Application of several botanical phytochemicals in Agricultural crops have been found effective in inhibiting the growth of bacteria pathogens such as *Xanthomonas* (Bajpai *et al*., 2010).Certain essential oils obtained from plants stand out as better antibacterial agents than the commonly used synthetic chemical antibacterial agents against plant pathogenic bacteria like *Xanthomonas* species (Bajpai *et al*., 2010; Gyorgyi *et al*., 2004; Nguefack *et al*., 2005). Results of this study showed that the disease incidence and severity of bacterial spot *V. unguiculata* were significantly reduced by the application of plant extracts used as bio-pesticides compared to untreated control. Almost all plant extracts used significantly ($P \leq 0.05$) reduced disease severity and incidence on leaves in the two years trials. Plant species like *Gongronema latifolium, Vernonia amygdalina* and *Ocimum gratissimum* have been reported to be promising species as crop protection biopepticides. (Stoll, 2000; Opara and Wokocha, 2008). These effects have been attributed to the peptides, alkalonoids, essential oils, phenols and flavonols which are major components in these plants (Okigbo and Igwe, 2007). These medicinal properties exert bacteriostatic and bacteriocidal effects on some bacteria. Prasad and Alankararao (1987) evaluated the antimicrobial effects of essential oils of fine species *Ocimum.* All the samples showed antibacterial activity against gram positive and gram-negative bacteria. Organic extracts applied to seeds and liquid extracts from composed material sprayed on leaves, have been found to restrain the development of fungal leaf pathogens (Emechebe and Alabi, 1997).

However, the percentage leaves infected and severity was particularly lower in plant treated with *V. amygdalina, G. latifolium* while *O. gratissimum* extracts substantially reduced incidence and severity of disease. This confirms the observations that many plant products contain anti-bacterial constituents that have potentials to control plant diseases. (Emechebe and Alabi 1997; Enikuomehim and Peter, 2002; Balm, 2003; Amadioha, 2003; Opara and Wokocha, 2008; Okigbo, 2009).

The consistent best performance of *V. amygdalina, G. latifolium* and *O. gratissimum* was observed as good antibacterial agent and that *V. amygdalina, G. latifolium* gave comparable reduction of *Xanthomonas axonopodis* pv *vignicola* this is in agreement with the work of Onuorah and Orji (2015). Consistently suppressed the mycelia growth of *P. sorghina* of *Telfairia occidentalis* Hook F. they also suppressed leaf spot disease and enhanced fresh leaf and pod yield. These results agrees with previous works associated with bacterial and fungal growth inhibition and disease suppression with a variety of plant bioactive compounds (Oluma and Elaigwe, 2006). Some of these compounds: alkaloid, polyphenols, biurates, saponins, terpernoids have been reported in all plants used in this study (Onuorah and Orji, 2015). Similarly, the biotoxic activity of *G. latifolium* against *Colletotrichum* isolate from tomato is attributable to alkaloids, saponins, and tannins (Onuorah and Orji, 2015).
Amadioha (2003) reported that *O. gratissimum* reduced the radial growth of *Rhizopus* spp. which cause avocado rots. This report was also seen in reduction of radial growth of *Cercospora* spp and observed that *O. gratissimum* and *A. ciliata* inhibited the radial growth of *Collectrichum* spp and compared favorably with benlate fungicide. Bdliya and Dahiru (2006) reported that in the field of potato plant extracts were found effective in treatment of potato rot caused by *Erwinia carotovora*.

Results of laboratory experiment showed that bacterial isolate from the infected leaves of *V. unguiculata* was a gram negative rod, motile with a single polar flagellum, catalase positive and oxidase negative and production of yellow colonies on nutrient agar. Based on the above characteristics description it was identified as *Xanthomonas axonopodis* (Bradbury, 1970; 1986; Buchaman and Gibbsons, 2004; Asuquo and Opara, 2016).

Results of pathogenicity test showed that symptoms found on disease *V. unguiculata*in in the field was also observed in inoculated seedling during the Pathogenicity test in pots after seven days. The bacterial spot symptoms observed in this study is in line with the reports about the leaf spot disease by previous researchers (Bradbury, 1970; 1986; Buchaman and Gibbsons, 2004; Asuquo and Opara, 2016).

Results of the field experiment showed that there was no significant difference within all plant extracts used in the study on growth and yield parameters but was

significant difference (P≤0.05) between the extracts and the untreated control as well as in diseases incidence and severity.

Conclusion

This study showed that plant extracts such as as *V. amygdalina*, *G. latifolium* and *O. gratissimum* can be used by resource poor farmers in control of bacterial leaf spot of vegetable cowpea in the field. It is viewed as an important edible vegetable crop within the rainforest and indigenous to South Eastern part of Nigeria. They contain antibacterial compounds such as saponins and flavonoids that can be utilized to prepare potential phyto-bactericides for the control of bacterial spot of *Vignicola* sdd. It showed that use of these plant extracts has the potentials to control bacterial spot disease at less or no cost. This kind of low-cost biological approach would be economically viable and ecosystem friendly which also provides alternative method for the elimination of *V. Unguiculata* diseases and can be easily recommended to small scale farmers in Nigeria. The extracts are also accessible to farmers as well as readily sourced locally. This research also recommends use of plant extracts to improve crop growth.

Table 1: Effect of Plant Extracts on Growth Parameters of Vegetable Cowpea Varieties in the 2017 Cropping Season

Var.	Plt. Ht. (cm)				No. Br.				No. Lf				S. Dia. (cm)			
	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT
BLACK	28.67	35.83	14.00	5.67	4.00	5.67	1458	4.02	4.08	4.67	14.50	2.83	0.59	0.67	0.76	0.66
BROWN	22.00	25.67	3.67	9.42	3.67	4.92	9.17	9.17	4.83	5.58	8.33	10.00	0,55	0..72	0.52	0.50
IFE BROWN	29.75	35.00	5.42	6.17	5,42	6.11	12.83	9.33	4.75	5.58	7.33	9.17	0.68	0.75	0.63	0.58
IFE 143	29..75	28.33	4.49	5.58	4.92	5.58	15.08	6.50	5.25	6.83	17.17	4.42	0.53	0.53	0.78	0.61
LSD (P≤0.05)	**8.67**	**5.83**	**4.00**	**5.67**	**4.00**	**5.67**	**9.58**	**6.92**	**4.31**	**4.42**	**5.12**	**5.13**	**0.25**	**0.25**	**0.51**	**0.42**

Legend: Plt.Ht.=Plant height in cm, No. Lf= Number of leaves, No.Br=Number of branches, S.Dia.=Stem diameter in cm, Dis. Inc. (%)=Percentage Disease incidence, Dis. Sev.=Disease severity, No.Pod =Number of pods, 1000Sd. Wt.=1000 Seed Weightt, T/ha = Tons of seed per Hect are, Var.= Variety, VA= *Vernonia amygdalina,* GL=*Gongronema latifolium,* OC=*Ocimum gratissimum,* CT= Control.

Table 2: Effect of Plant Extracts on Disease and Yield Attributes of Vegetable Cowpea 2017 Cropping Season

Var.	Dis. Sev.				% Dis. Inc.				No. Pod/plt				1000 Sd.Wt.(g)/Plt				Sd Wt. (t/Ha.)			
	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT
BLACK	**4.00**	**12.58**	**12.35**	**29.00**	**6.25**	**6.03**	**5.03**	**37.25**	**6.51**	**9.06**	**8.61**	**5.04**	**9.58**	**12.76**	**11.51**	**4.09**	**8.42**	**28.14**	**24.26**	**6.61**
BROWN	**5.00**	**7.67**	**6.23**	**29.08**	**8.50**	**6.00**	**4.50**	**25.33**	**6.42**	**8.16**	**7.29**	**1.70**	**8.72**	**1232**	**10.47**	**4.40**	**9.16**	**20.12**	**20.87**	**5.77**
IFE BROWN	**6.00**	**6.07**	**7.58**	**19.83**	**10.4**	**6.08**	**5.58**	**35.75**	**5.36**	**7.55**	**6.97**	**4.00**	**9.46**	**10.19**	**13.12**	**4.54**	**8.83**	**19.47**	**19.67**	**4.92**
IFE 143	**3.99**	**2.00**	**3.67**	**8.17**	**13.6**	**6.20**	**5.25**	**27.33**	**5.22**	**7.12**	**6.51**	**1.80**	**9.54**	**9.77**	**12.16**	**4.12**	**9.72**	**30.33**	**17.52**	**6.43**
LSD (P≤0.05)	**0.21**	**0.84**	**2.86**	**0.16**	**0.44**	**1.80**	**1.79**	**18.90**	**0.89**	**0.97**	**0.95**	**0.50**	**1.24**	**1.29**	**3.72**	**0.56**	**1.30**	**4.76**	**4.97**	**0.94**

Legend: Plt.Ht.=Plant height in cm, No. Lf= Number of leaves, No.Br=Number of branches, S.Dia.=Stem diameter in cm, Dis. Inc. (%)=Percentage Disease incidence, Dis. Sev.=Disease severity, No.Pod =Number of pods, 1000Sd. Wt.=1000 Seed Weightt, T/ha = Tons of seed per Hect are, Var.= Variety, VA= *Vernonia amygdalina,* GL=*Gongronema latifolium,* OC=*Ocimum gratissimum,* CT= Control.

Table 3: Effect of Plant Extracts on Growth Parameters of Vegetable Cowpea Varieties in the 2018 Cropping Season

Var.	Plt. Ht. (cm)				No.Br.				No. Lf				S. Dia (cm)			
	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT
BLACK	32.08	10.67	4.51	4.00	3.33	6.08	7.58	3.25	4.08	17.17	46.08	12.13	0.43	0.62	0.66	0.47
BROWN	19.42	11.58	4.57	3.67	2.92	7.67	9.17	3.92	4.83	10.00	38.75	6.62	0.39	0.42	0.50	0.56
IFE BROWN	26.42	11.17	4.58	5.42	5.00	10.17	12.83	4.25	4.75	9.17	21.17	4.58	0.37	0.54	0.58	0.47
IFE 143	25.92	11.50	4.63	4.92	4.50	13.67	15.25	4.50	5.25	4.42	6.92	3.09	0.31	0.58	0.61	0.59
LSD (P≤0.05)	**21.74**	**0.74**	**0.59**	**2.17**	**2.24**	**1.10**	**0.55**	**0.60**	**0.58**	**1.07**	**4.71**	**0.59**	**0.06**	**0.25**	**0.27**	**0.24**

Legend: Plt.Ht.=Plant height in cm, No. Lf= Number of leaves, No.Br=Number of branches, S.Dia.=Stem diameter in cm, Dis. Inc. (%)=Percentage Disease incidence, Dis. Sev.=Disease severity, No.Pod =Number of pods, 1000Sd. Wt.=1000 Seed Weightt, T/ha = Tons of seed per Hectare, Var.= Va riety, VA= *Vernonia amygdalina,* GL=*Gongronema latifolium,* OC=*Ocimum gratissimum,* CT= Control.

Table 4: Effect of Plant Extracts on Disease and Yield Attributes of Vegetable Cowpea Varieties during 2018 Cropping Season

Var.	Dis. Sev.				% Dis. Inc.				No. Pod/plt				1000 Sd. Wt.(g)/Plt				Seed Wt. (t/Ha.)			
	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT	VA	GL	OC	CT
BLACK	4.00	6.58	10.42	11.25	27.50	5.17	4.33	27.33	8.25	5.92	10.08	4.75	29.33	25.75	36.59	6.49	46.08	47.92	12.13	4.42
BROWN	9.75	7.25	10.42	11.17	20.99	5.00	3.75	25.75	8.00	5.83	10.33	4.67	28.00	22.50	26.59	6.35	38.75	32.08	16.62	6.39
IFE BROWN	8.25	5.83	10.42	11.17	26.42	5.25	4.50	30.25	8.41	5.75	10.25	4.58	26.83	22.92	26.51	6.32	21.17	20.08	14.58	4.41
IFE 143	3.42	5.83	10.42	10.67	26.42	5.25	4.50	30.25	8.33	5.42	10.08	4.92	28.75	23.33	6.73	16.35	16.92	12.63	13.09	2.14
LSD (P≤0.05)	**1.29**	**1.47**	**0.55**	**0.65**	**19.08**	**2.23**	**2.17**	**19.00**	**1.02**	**0.43**	**1.02**	**0.58**	**3.28**	**3.28**	**0.58**	**10.20**	**1.04**	**3.41**	**2.76**	**1.78**

Legend: Plt.Ht.=Plant height in cm, No. Lf= Number of leaves, No.Br=Number of branches, S.Dia.=Stem diameter in cm, Dis. Inc. (%)=Percentage Disease incidence, Dis. Sev.=Disease severity, No.Pod =Number of pods, 1000Sd. Wt.=1000 Seed Weightt, T/ha = Tons of seed per Hect are, Var.= Variety, VA= *Vernonia amygdalina,* GL=*Gongronema latifolium,* OC=*Ocimum gratissimum,* CT= Control.

Table 5: Morphological and Biochemical Characterization of bacterial leaf spot pathogen of *V. unguiculata* L. Walp

Test	Result
Morphological characteristics	
Gram	-ve
Motility	+ve
Spore	-ve
Biochemical characteristics	
Catalase	+ve
Oxidase	+ve
NO_3	-ve
H_2S	+ve
Indole	+ve
MR	+ve
V.P	+ve
Urease	-ve
Glucose	+ve
Lactose	-ve
Maltose	+ve
Manitol	-ve
Xylose	+ve
Sucrose	+ve

Legend; - = Negative, + = Positive

Table 6: Soil Analysis of Experimental Site

Physical properties	Result
Sand	72.79%
Silt	10.40%
Clay	16.81%
Texture	Sandy loam
Chemical properties	
PH	5.40
Nitrogen	0.042mgkg-1
Organic matter	1.31mgkg-1
Organic carbon	1.20mgkg-1
Exchangeable bases	
Calcium	2.38cmol kg-1
Magnesium	1.22cmol kg-1
Potassium	0.08cmol kg-1
Sodium	2.51cmol kg-1
Exchangeable acidity	1.32cmol kg-1

References

Adu-Dapaah, H., Afun J.V.K., Asumadu, H. Padi, F and Gyasi-Boakye, (2005). Cowpea production guide. C. Oti-Boateng (ed) 42 pp.

Ajibade, S. R. and Amusa, N. A. (2001). Effects of Fungal diseases on some Vegetable cowpea lines in the humid environment of South-Western Nigeria. *Journal Sustainable Agricultural Environment*, 3: 246-253.

Akpan, A. U. (2014). Effect of planting Density and variety on the vegetative growth and fresh pods yield of two Vegetable cowpea varieties (*Vigna unguiculata* (L) Walp.) in South Eastern Nigeria. *Journal of Science of Agriculture, Food Technology and the Environment* 13:74-78.

Allen, D. J. and Lenne, J.M. (1998). Diseases as constraints to production of legumes in agriculture. In Pathology of Food and Pasture Legumes. Allen, D. J. Lenne J. M. (Eds.). CAB International, Wallingford, UK. pp. 1-61.

Allen, D. J., Thottappilly, G., Emechebe, A. M. and Singh, B. B. (1998). Diseases of vegetable cowpea. In Pathology of Food and Pasture Legumes of Vegetable cowpea. Allen DJ, Lenne JM (Eds.). CAB International, Wallingford, UK. pp. 267-324.

Amadi, J. E. (1995). Chemical control of Cercospora leaf bacterial spot disease of Vegetable cowpea (*Vigna unguiculata* (L.) Walp.). *Agrosearch.* 1: 101-107.

Amadioha, A. C. (2003). Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. *Acta Phytopathologia,* 38:259-265

Amadioha, A. C. and Obi, V. I. (1999). Control of anthracnose disease of *Vigna unguiculata* by *Cymbopogon citrates* and *Ocimum gratissimum. Acta. Phytopathol. Entomol. Hungerica,* 34 (1-2): 85-89.

Amatobi, C. I. (2000). Cashew plant crude extract as a

promising insecticide in cowpea insect pest management. Abstracts of paper and poster presentations, World Cowpea Res. Conf. 111, IITA, Ibadan, Nigeria, 4-7 September, 2000, 11pp.

Ano, A. O., and Ubochi, C. I. (2008). Nutrient composition of climbing and prostrate *Vigna unguiculata* accessions. *African Journal of Biotechnology.* 7(20): 3795-3798.

Asuquo, A. A. and Opara, E. U. (2016). Application of Some Management Strategies on Leaf spot and Fruit Rot Diseases of Watermelon (*Citrullus Lanatus*) in South Eastern Nigeria. *International Journal of Research in Agriculture and Forestry*, 4(2): 29-40.

Awurum, A. N. and Enyiukwu, D. N. (2013). Evaluation of seed-dressing potentials of phytochemicals from *Carica papaya and Piper quinensence* on the germination of Vegetable cowpea (*Vigna unquiculata* (L.) Walp) seeds and Incidence of the seed-borne fungi. *Continental Journal of Agricultural Science*, 7 (I):29-35.

Awurum, A. N., Enyiukwu, D. N. and Uchegwu, P. O. (2014). Effectiveness of duration of storage and phytochemicals in the management of seed-borne mycoflora of stored cowpea (*Vigna unquiculata* (L.) Walp) Seeds. *Greener J. Agron. Forest. Hort*., 2(2):022-026.

Bajpai, V. K., Dung, N.T., Suh, H. J. and Kang, S. C. (2010). Antibacterial activity of essential and extracts of *Cleistocalyx operculatus* buds against the bacteria of *Xanthomonas spp. Journal Am. Oil Chem. Soc*. 87: 134-1349.

Balm, A. (2003). Neem: A therapeutic for all seasons, current science, 82 (11) June pp 1304.

Bankole, S. A. and Adebanjo, A. (1996). Biocontrol of brown blotch of *Vigna unguiculata* caused by *Colletotrichum truncatum* with *Trichoderma viride*. *Crop Prot.*, 15: 633-636.

Bdliya, B. S, and Dahiru, B. (2006). Efficacy of some plant extracts in the control of potato tuber soft rot caused by *Erwinia carotovora ssp carotova. J plant prot res*., 46 (3): 285-294.

Begum, J., Yusuf, M., Chowdhury, U. and Wahab, M. A. (1993). Studies on essential oils for their antibacterial and antifungal properties. Part 1. Preliminary screening of 35 essential oils. *J. Sci. Ind. Res*., 28: 25-33.

Bradbury, J.F. (1970). Isolation and preliminary study of bacteria from plants, Rev. Plant Pathology. *Global Advanced Research Journal of Agricultural Science*, 1(2): 49:213-218.

Bradbury, J.F. (1986). Erwinia. Guide to Plant Pathogenic Bacteria. CAB International Mycological Institute, New York, pp: 67. [25]

Buchaman, R.E. and Gibbon, N.E. (2004). Bergy's Manual for Determinative bacteriology, 8th Edition,Williams and Watkins Ltd, UK.

Brink, B. (2013). Urease Test Protocol. American Society for Microbiology Microbe Library, 763 pp.

Cheesebrough, M. (2000) District Laboratory Practices in Tropical Countries Secod Edition Part 1 Cambridge University Press, Pp. 206-454.

Cheesbrough M. (2002). District labour practices in tropical countries (part 2). Cambridge University. press. Pp 137-139.

Collins, C. H., Lyne P. M., Grange J. M. and Falkinham J. O. (2004). Identification methods. In: Collins C. H., Lyne P. M., Grange J. M., Falkinham J. O., editors. Colins and Lyne's Microbiological Methods. 8th ed. Arnold; P. 89-109.

David, H. G. (2005). Management of *Xanthomonas* leaf blight of onion with a plant activator, biological control agents and copper bactericides PL DIS. 89(6):631-639.

Dawson, J. R., Johnson, R. A. H., Adams, P. and Last, F. T. (1965). Influence of steam/air mixtures, when used for heating soil, on biological and chemical properties that affect seedling growth. *Ann of Appl. Biol.*, 56:243-251.

Duke, P. B. and Jarvis, J.D. (1972). The catalase test-a cautionary tale. *J. Med. Lab Technol.*, 29(2):203-204.

Effraim, I. D., Salami, H. A. and Osewa, T. S. (2000). The effect of aqueous leaf extract of Ocimum gratissimum on haematological and biochemical parameters in rabbits. *Afr. J. Biomed. Re*s., 175-179.

Eleyinmi, A.F. (2007). Chemical composition and antibacterial activity of Gongronema latifolium. *J. Zhejiang Universal Sci.*, 8: 352-358.

Emechebe, A.M. and Alabi, O. (1997). Evaluation of Aqueous extracts of parts of some plant for the control of cowpea diseases at Samaru croppin scheme meeting: Report on legume and oilseeds Research Programme pp.77.

Emechebe A. M. and Lagoke S. T. O. (2002). Recent advances in research on cowpea diseases. In: Fatokun CA, Tarawali SA Singh BB, Kormawa PM, Tamo M(eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference 111 held at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. IITA Ibadan, Nigeria. Pp. 94-123.

Emechebe, A. M. and McDonald, D. (1979). Seed-borne pathogenic fungi and bacteria of Vegetable cowpea in Northern Nigeria. PANS. 25: 401-404.

Emechebe, A. M. and Shoyinka, S. A. (1985). Fungal and bacteria diseases of Vegetable cowpea in Africa. In *Vigna unguiculata* Research, Production and Utilization. Singh SR, Rachie KO (Eds.), John Wiley and Sons, Chichester, UK. Pp. 173-192.

Emechebe, A.M. and Florini D. A. (1997). Shoot and pod diseases of *Vigna unguiculata* induced by fungi and bacteria. In Advances *Vigna unguiculata* Research. Singh BB, Mohan DR (Eds.), Pp. 176-192.

Emechebe, A. M. and Lagoke, S. T. O. (2002). Recent Advances in Research on cowpea diseases, in: Challenges and opportunities for enhancing sustainable cowpea production. C. A. Fatokun, S. A. Tarawali, B. B. Singh, P. M. Kormawa and Tamo (Eds). International Institute of Tropical Agriculture (IITA), Ibadan, Pp.94-123.

Enikuomehin, O.A. and Peter, O. T. (2002). Evaluation of crude extracts from some Nigerian plants for the

control of field disease of sesame (*Sesamum indicum* L). *Trop.Oilseeds J.*, 7:84-93

Ganiyu, S., Popoola, A., Owolade, O. and Fatona, K. (2017). Control of common bacterial blight disease of Vegetable cowpea (*Vigna unguiculata* (L.) Walp) with certain plant extracts in Abeokuta, Nigeria. *Journal Crop Improvement*, 31: 280–288.

Gee, G. W. and Bauder J. W. (1986). Particle size analysis. In:Wute A. (ed.): method of soil analysis, Partl: physical and mineralogical methods, Soil Science Society of America, Maidson, Wisconsin, 383-411.

Hernandez, N.E., Tereschuk, M.L. and Abdala, L.R. (2000). Antimicrobial activity of flavonoids in medicinal plants from Tafi del Valle (Tucuman, Argentina). *J Ethnopharmacol*., 73(1-2):317-322.

Jones J. B., Bouzar, H., Stall, R. E., Robert, P. D., Bowen, B. W., Sunberry, D., Strewer, P. M., and Chin, J. (2000) Systematic analysis of (*Xanthomonas* spp.) associated with pepper and tomato lesions. *International Journal of Systematic and Evolutionary Microbiology*, 50:11-12.

Karuna, K. and Khan, A. N. A. (1993). Effect of plant extracts on *Pseudomonas solanacearum* causing wilt of tomato plants. *Indian phytopathol*, 47:326.

Kiran Kumar, (2000), Studies on the effect of botanicals on important plant pathogenic bacteria M.sc. (Agric.) Thesis, Uni. Agric. Sci., Bangalore, Karnataka (India). 240pp

Latunda-Dada, A. O. (1991). The use of *Trichoderma koningii* in the control of wed blight disease caused by *Rhizoctonia solani* in the foliage of cowpea *Vigna unguiculata*. *J. Phytopatho*., 133:247-254.

Latunde-Dada, G. O. (1993). Iron contents and some physical components of twelve *Vigna unguiculata* varieties, *Int. J. Food Sci. Nutr*. 43: 193-197.

Mac Faddin, J. F. (2000). Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams and Wilkins, Philadelphia, PA. 340pp

Morebise, O., Funso, M.A., Makinde, J.M., Olajide, O.A. and Awe, E.O. (2002). Antiinflamatory property of the leaves of *Gongronema latifolium. Phytother Res*., 16(1):75-77.

Nelson, D. W. M. and Somners, L. E. (1982). Total Organic Carbon and Organic matter. In" Methods of soil Analysis, Part 2 Miller, R. H. and Keany, D. R. (eds). Amer. Society of Agronomy, Madison, U.S.A. Pp. 539-580.

Nguefack J., Somda I., Mortensen C.N. and Am Zollo P.H (2005). Ealation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (*Oryza sativa* L.). *Seed Science Technology*.33:397-407.

Ogunwanle, I. A., Walker T. M. and Setzer, W. N. (2007). A review of aromatic herbal plants of medicinal importance from Nigeria. *Nat. Prod. Comm*., 2(12): 1311-1316.

Okechukwu, R. U. and Ekpo, E. J. A. (2008). Survival of *Xanthomonas campestris pv. vignicola* in infected soil, cowpea seed and cowpea debris. *Trop Agric. Res and Extension*, 11: 43-48.

Okigbo, R. N., Igwe, M. (2007). The antimicrobial effects of *Piper guineense*; uziza and Phyllantus amarus ebe-benizo on Candida albicans and *Streptococcus faecalis*. Acta Microbiologica .et. Immunologica. Hungarica. 54 (4): 353-366.

Okigbo, R. N. (2009). Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi State, Nigeria. *American Eurassian Journal of sustainable Agriculture*, 3(3): 407-409.

Okigbo, R. N., Ajale, A. N. (2005): Inhibition of some human pathogens with the tropical plant extracts Chromolineena odorata and Citrus aurantifolia and some antibiotics. *Int. J. Mol. Med. Adu Sci*., 1:34-40

Okpareke, A. M., Dike, M. C. and Amatobi, C. I. (2001). The potential for controlling post flowering insect pests of cowpea *Vigna unguiculata* L Walp using cashew *Anacardium ocidentale* L. product extracts, paper presentation, 32nd Ann. Conf. ESN, National Library Auditorium, Kaduna, Nigeria, 488pp

Oluma, H. O. A and Elaigwe, M. (2006). Antifungal activity of extracts of some medical plants against *Macrophomina phaseolina. J. Bot.,* 19(1): 121-128.

Onuorah, S. and Orji, M. U. (2015). Fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria. *Univ. J. Microbiol. Res*., 3(2):11-16.

Opara, E. U. and Wokocha, R. C. (2008). Efficacy of some plant extract on the invitro and invivo control Xanthosomas *campestris pv* vesicatoria. *Medwell Agric Journal*, 3 (3):163-170

Prasad, R. and Alankararao, A. (1987), In vitro antimicrobial screening of Indian essential oils part 1. *Ocimum* spp. J. Sci. Res., 9:79.

Quin, F. M. (1997). Importance of *Vigna unguiculatain* Advances in *Vigna unguiculata* Research. B.B. Singh, K.E. Dashiell, D.R. Mohan Raj and L.E.N. Jackai (Eds.), Pg. X-Xii. Printed by Colcorcraft, Hong Kong, p. 375

Rachie, K. O. (1985). Introduction. In Vegetable cowpea: Research, Production and Utilization. Singh, S.R. and K.O. Rachie (eds). John Wiley. 1:1-42.

Roades, J. D. (1982).Cation Exchange Capacity. In Page, A. L. Miller, R. H. and Keeney, D.R. (eds.) Methods of soil analysis. Part 2 Agron. Monogr. ASA, SSSA, Madison, W.I. USA.

Scheneider, R. W., William, R. J. and Sinclair, J. B. (1976). Cercospora leaf spot of vegetable cowpea: Models for estimating yield loss. *Phytopathology,* 66: 384-388.

Shah, R., Bhatnager, M. K. and Dashora P. K. (1991), Control of bacterial blight of cowpea, Indian *J. Mycol. PL. Pathol.*, 20(3): 275-276.

Sikirou, R. (1999) Epidermiological investigations and development of integrated control methods of bacterial blight of Cowpea caused by *Xanthomonas Campestris pv. vignicola*. PHD thesis. University of Gottingen, Germany. pp. 218

Snell, J. J. S., Brown, D. F. J. and Roberts, C. (1999). Quality Assurance Principles and Practice in the Microbiology Laboratory. London: Public Health Laboratory Service. Pp.147-148.

Stoll, G. (2000). Natural Crop Protection in the Tropics and Sub-Tropics. AGRECOL, Switzerland, 1998; Pp.188. 11. Stoll G. Natural Crop Protection in the Tropics and Sub-Tropics Letting information come to life 2nd Edn., Margraf Verlag. Germany, 101-139.

Yayock, J. Y., and Asenime, E. (1977). Effects of Fertilizer, Plant Density and Sowing Date on Yield and Other Characters of Cowpea (*Vigna unguiculata* (L) Walp) in Northern Nigeria, Savanna. Miscellaneous Paper, 69. P.17.